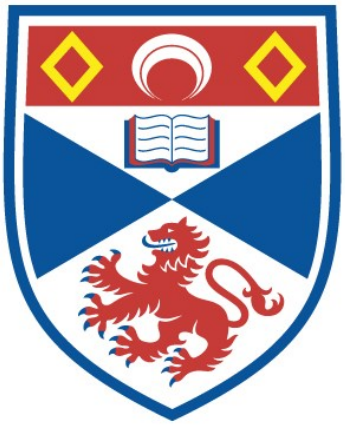
LIGHT, PHOTOSYNTHESIS AND GROWTH OF SUBLITTORAL MACROALGAE IN BRITAIN AND THE MEDITERRANEAN

William A. A. Robertson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1976

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Abstract

Physiological studies were made of 23 species of red seaweeds (Rhodophyta) and 2 species of green seaweeds (Chlorophyta) in Sicily and at various sites in the British Isles. In Britain, these algae formed part of an "underflora" beneath the canopy of the dominant sublittoral "kelp forest" of Laminaria hyperborea (Phaeophyta). In the Mediterranean, the algae studied formed a turf composed largely of green and red species. In Britain, a standing crop of non-laminarian species of 140g dry weight m^{-2} was recorded at 5m depth, and approximately half of this at 12m; the crops were about 4 and 12% respectively of the total biomass per m^2. At Sicily (Ganzirri, Straits of Messina) a maximum crop of 1360g (dry weight) m^{-2} was recorded at 15m depth.

Radioactive tracer (^{14}C) and dissolved oxygen (Winkler) techniques were developed for use underwater to depths of 60m.

Photosynthetic rates measured under agitated incubation conditions were approximately twice the values obtained under static conditions. Rates of photosynthesis measured using the ^{14}C technique were generally high in shallow algae incubated in situ in Britain, e.g. 20 µg C cm^{-2} h^{-1} for Porphyra umbilicalis at 0m depth; 11 µg C cm^{-2} h^{-1} for Rhodymenia palmata, 3m depth. Deeper algae had lower in situ rates, e.g. Delesseria sanguinea, 3.1 µgC cm^{-2} h^{-1} and Phycodrys rubens, 1.8 µgC cm^{-2} h^{-1}, both at 18m depth. At Ganzirri, using the ^{14}C method, Porphyra umbilicalis attained a rate of 18 µgC cm^{-2} h^{-1} at 4.5m and Pseudolithophyllum expansum

4.7 gC cm^{-2} h^{-1} at 60m depth.

Rates of photosynthesis were strongly reduced by the reduction of irradiance by the water column. Reduction of rates was even more strongly influenced by self-shading in algal stands, Dilsea reaching a photosynthetic efficiency of 8% beneath a L.hyperborea canopy at 4m depth.

In general, deep-growing algae were found to be "shade-adapted" (low maximal photosynthetic rates, high-efficiency at low irradiance) and shallow-growing algae were "sun-adapted" but there were notable exceptions. Adaptation occurred within single species.

At the deep sites, green algae had photosynthetic rates as high as, or higher than, coexisting red species, suggesting that the red algae had no simple intrinsic photosynthetic advantage conferred by their accessory pigments.

Deep specimens of red species exhibited photoinhibition of photosynthesis and photodestruction of pigments when incubated in surface solar irradiance of N 40 J cm^{-2} PAR. This was noted in such species as Delesseria sanguinea, Phycodrys rubens, Peyssonelia sp., Pseudolithophyllum expansum.

Few species studied were below 24-hour compensation point during the summer months and high irradiances of the studies. Few of the deeper algae, however, were operating at or above saturation for any significant length of time. They were thus generally operating at their own maximal efficiencies for most of the time.

Light, Photosynthesis and Growth of Sublittoral Macroalgae in Britain and the Mediterranean

by

William A.A. Robertson

Thesis presented for the degree of

Doctor of Philosophy

Gatty Marine Laboratory
and Department of Botany
University of St. Andrews

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DECLARATION

I hereby declare that the following thesis is based upon work carried out by me, that the thesis is my own composition, and that it has not been previously presented for a higher Degree.

The research was conducted at the Garry Marine Laboratory, University of St. Andrews, and was supervised by Dr. E.A. Drew.

CERTIFICATE

I certify that William A.A. Robertson has spent twelve terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967, No. 1., and that he is accordingly qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

CURRICULUM VITAE

I was educated at the High School of Dundee and the University of St. Andrews where, in 1970, I graduated with first class honours in Botany. Thereafter I held a SRC post-graduate studentship of three years' duration, and a St. Andrews University research scholarship of one years' duration, during which time the studies for this thesis were carried out.

ACKNOWLEDGEMENTS

I wish to express my gratitude to my supervisor, Dr. E.A. Drew for his continued interest in the work, for much helpful discussion and for indispensible practical assistance during diving operations in Britain.

Generous thanks are also due to Mr. J.D. Robinson without whose help none of the hazardous diving operatings at Ganzirri would have been possible.

I must sincerely thank Mr. P.R. Balch for useful discussions and for translating my ideas into "Algol-W" and thus producing the computer programmes; also Dr. J.F. Ireland, for help with the spectrophotometry, and for much helpful discussion.

Thanks are also due for the indispensible cooperation of the numerous personnel at the various field sites; Professor Bolognari and his staff, of the University of Messina; the organisers and members of the Islands of County Kerry Expedition, 1973; Mr. H.T. Powell and Mr. M. Picken of the Scottish Marine Biological Association laboratory at Dunstaffnage; many members of various St. Andrews University diving and biological expeditions to Durness.

I also wish to thank the technical staff of the Gatty Marine Laboratory and Botany Department for help throughout the work and writing-up.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

To the casual observer, the marine macroalgae, the seaweeds, form a conspicuous part of the marine flora, principally because they are macroscopic, and generally occur in abundance at the land-sea interface frequented by man. In fact, although their productivity is locally high, the seaweeds account for only a small part of the total plant biomass in the sea, the remainder being composed of the phytoplankton, which abound in the surface waters of wide areas of the oceans. A great proportion of our knowledge of the seaweeds was gained in the late 19th and early 20th centuries, from studies which were of a descriptive taxonomic or broadly ecological nature. In more recent times, much information has accumulated concerning the physiology and biochemistry of the algae as a whole, although much of this work has been carried out due to the convenience of certain algae, mainly unicells, as experimental organisms. Of the physiological studies on macroalgae, most have been conducted in the laboratory, due to the difficulties associated with conducting experiments in the field. In the present work, an attempt has been made to study the physiology of certain macroalgae, red algal species in particular, in situ as well as in the laboratory, and so to correlate the physiology with the observed algal distributions in the sea.

The marine macroalgae are strikingly different in appearance from the majority of other multicellular photosynthetic organisms due to their varied colours, attributable to differing combinations of photosynthetic pigments. All three of the major macroalgal divisions contain one or more forms of chlorophyll and a proportion of the yellow accessory pigments, the carotenoids.

In the Chlorophyta, the green algae, this pigment combination alone provides the characteristic colouring, similar to that of the higher plants. In the Phaeophyta, or brown algae, another group of accessory pigments, the xanthophylls, give the characteristic colour, and in the Rhodophyta, the red algae, the red colouring is given by a unique group of water-soluble accessory pigments, the phycobilins (also present in the blue-green algae, the Cyanophyta). Although all three divisions are well represented in the littoral zone, it was early recognised (Oersted 1844, cited by Levring 1947) that there were more species of Chlorophyta in the littoral zone than in the sublittoral, more species of Rhodophyta in the sublittoral than the littoral, whilst the Phaeophyta had a fairly uniform distribution throughout the whole shore profile. In temperate seas in fact, both the littoral and sublittoral zones are generally dominated by a relatively small number of species of the Phaeophyta. This domination relegates the other two groups to the position of an "underflora" community, typified by the profuse, predominantly red algal, epiphytic and lithophytic community festooning the Laminaria hyperborea "forest" of the sublittoral zone. In the clearer waters of warmer latitudes, however, where light penetrates deeper, and is of a different spectral quality, than in the temperate zone, e.g. the Mediterranean (see Larkum et al. 1967) and in the Pacific (see Gilmartin 1960), green algae may dominate the sublittoral communities. In both temperate and warmer water zones however, the lower limit of the photic zone is generally dominated by a small number of species of the Rhodophyta.

Considering again the pigmentation of the Rhodophyta, there are two principal forms of phycobilin, or "biliprotein", namely the red phycoerythrin which absorbs green light, largely of those wavelengths generally designated as occupying the "window" of the chlorophyll spectrum, and the blue-coloured phycocyanin (predominant pigment of the blue-green algae) which absorbs orange light. Although both pigment forms are widespread in

Rhodophyta, the relative quantities vary with habitat, large proportions of phycoerythrin being present in deep-growing specimens imparting their roseate colouring, and higher proportions of phycocyanin being present in littoral specimens, causing these to appear almost black in extreme cases. The absorption spectra of the pigments were investigated first by Sorby (1876). In the first studies of in vivo photosynthetic action spectra in red algae, Englemann (1883), using a motile aerobic bacterium to bioassay the evolution of oxygen from red algal filaments irradiated with a micro-spectrum, found that most photosynthesis occurred in green light. From this finding, and a knowledge of the well-known depth distribution of the algal divisions described above, Engelmann (1883) suggested that it was the inter-action between light quality and photosynthesis which was the principal factor in producing the underwater zonation of the macroalgae. In particular, he postulated that the Rhodophyta thrived at the lower limit of the photic zone because of their ability to utilise the green light there to greater advantage than their competitors, which lacked phycoerythrin. The idea was formalised by Gaidukov (1903) and termed the theory of "complementary chromatic adaptation" because it suggested that algae grew best in light of a colour complementary to that observed in their pigmentation. Confirmation that phycoerythrin was active in photosynthesis came from the work, using filtered light sources, of Wurmser (1923), Montfort (1936) and Levring (1947), but was first critically established by Haxo & Blinks (1950), using a monochromator and sensitive oxygen electrode to produce continuous photosynthetic action spectra of green, brown and red macroalgae. Their results showed that, in green algae, the action spectrum followed the absorption spectrum closely (see Figure 1.1A), except for a marked drop in quantum efficiency of chlorophyll at the extreme red end of the absorption spectrum already termed by Emerson et al. (1957) the "red drop".

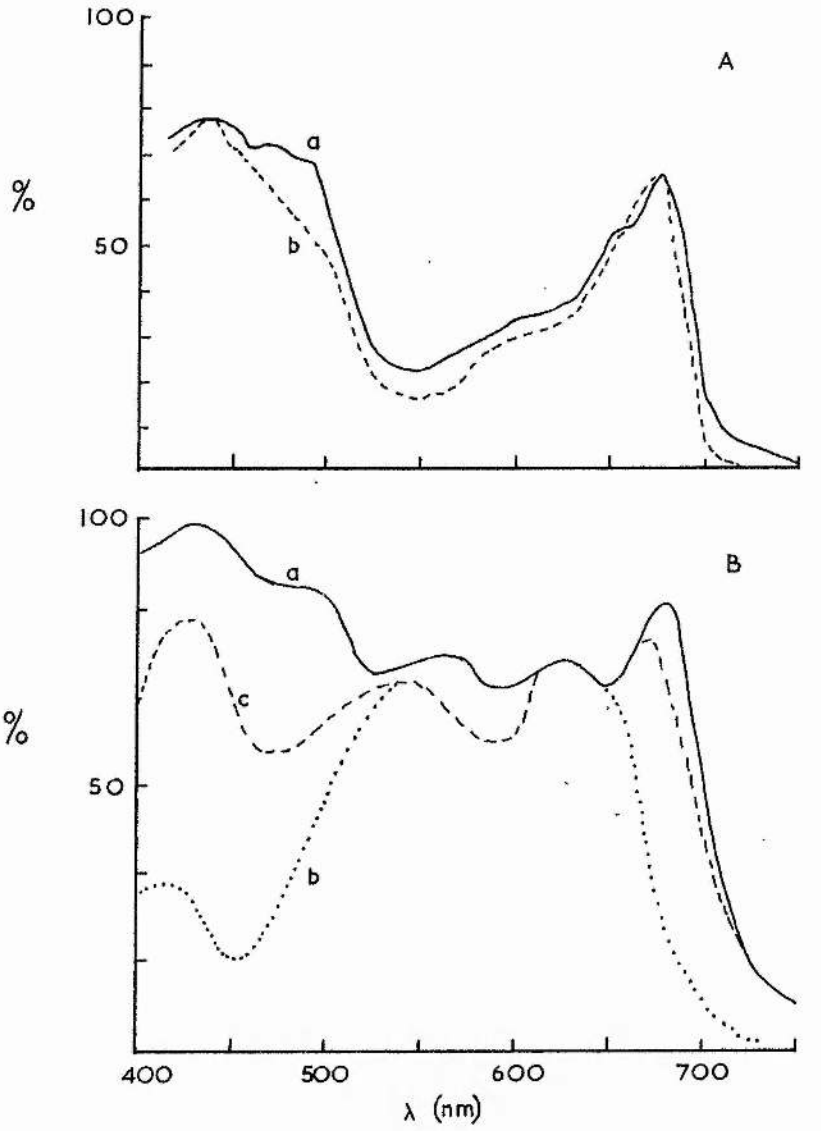


Figure 1.1. In vivo absorption (a) and photosynthetic action (b) spectra of green and red macroalgae; A, Ulva taeniata (Chlorphyta); B, Porphyra perforate (Rhodophyta), showing action spectrum without (b) and with (c) low level of supplementary green enhancing radiation of 546nm. (A from Haxo & Blinks 1950, B, from Fork 1963).

In red algae, however, Haxo & Blinks (1950) found that although photosynthetic activity was high in the green spectral region in accordance with the absorption by phycoerythrin, the quantum efficiency in the blue and red wavelengths was extremely low causing both blue and red "drops" even although absorption due to chlorophyll was quite appreciable in these spectral regions (Figure 1.1B curves a and b). Haxo & Blinks concluded that, for some reason, chlorophyll in red algae was "inactive",and this tended to strongly confirm the chromatic adaptation hypothesis, indicating that the red algae were indeed almost obligately adapted for life in green light, i.e. that prevailing underwater, especially in temperate coastal regions. However, Emerson et al.(1957) discovered that if, while carrying out a scan of the action spectrum of photosynthesis in Chlorella, they added a low level of supplementery light of a certain wavelength, the red drop of chlorophyll was eliminated; this became known as the "Emerson enhancement effect". Then, in 1963, Fork repeated some of the action spectrum experiments of Haxo & Blinks (1950) using pairs of wavelengths of light, as Emerson et al.(1957) had done, and found, that in a manner similar to the Emerson enhancement effect, the blue and red drops in red algae could be greatly diminished, yielding a photosynthetic action spectrum which was very much closer to the in vivo absorption spectrum (Figure 1.1B, curve c). Fork (1963) thus suggested "the inactive chlorophyll in red algae could better be termed unenhanced chlorophyll". Since the underwater spectrum is unlikely ever to be truly monochromatic within the photic zone, Fork's (1963) results suggest that enhancement effects probably occur in nature and thus the Rhodophyta probably utilise radiant energy in direct relation to their absorption spectrum. The original finding of Haxo & Blinks (1950) however, has proved tenacious in the literature and many reviews and reference works continue to propound the concept that chlorophyll is "inactive" in the red algae (e.g. Fogg 1968; Prescott 1969;

O'h Eocha 1971; Dixon 1973; Govindjee & Braun 1974). It should be mentioned that there is evidence which conflicts with the work of Fork (1963) in the results of Halldal (1964, 1967, 1974) who determined the photosynthetic action spectra of several red algal species using a somewhat unconventional monochromator system. Although the spectra were measured in the presence of a low level of "white" irradiance, photosynthetic activity in the blue and red light was very low indicating that the white light had no enhancing effect. Halldal has consistently argued the case for inactivity of chlorophyll in red algae, although he has made no reference either to the Emerson enhancement effect or to the results of Fork (1963). The question of the action spectrum of photosynthesis in the red algae must therefore remain somewhat incompletely answered at present.

In opposition to theories involving the spectral quality of light (i.e. the chromatic adaptation theory) Berthold (1882) and Oltmanns (1892) had suggested that the prime factor influencing the underwater colonisation of the algae might be quantity, or intensity of irradiance. The concept of limitation of plant growth by the limiting quantity of an essential factor was introduced in 1840 by von Liebig (see Odum 1959, p.88) in connection with the influence of soil nutrients on crop yields, Liebig's "Law of the minimum", but was first applied to the external control of photosynthetic rate by Blackman (1905) in his classic appraisal of "Optima and limiting factors". He characterised "conditions of supply of material or of energy" as the two prime factors limiting photosynthesis. Clearly, the increase in depth in the sea produced a progressive reduction in the quantity of the prime limiting factor, energy, or light, and the reduction in total quantity of algal biomass could be regarded as a response to this. However, this decline in quantity of energy with depth could be expected to produce, in addition to the concomitant decline in the total algal

population, a range of ecotypes differently adapted to the range of levels of irradiance. Land plants are known to exhibit adaptation to different levels of irradiance, so-called "sun-shade" adaptation. Such adaptation can occur phenotypically, due to environmental action upon individual plants on a short term basis, forming "ecotypes", or to whole plant taxa which have become genetically adapted to sun or shade habitats. In general, "sun plants" (e.g. many terrestrial plants and crops) attain high maximum photosynthetic rates at high levels of irradiance. "Shade" plants (e.g. "understorey" plants and many mosses) attain their maximum rates of photosynthesis at much lower irradiances, and very high irradiances may even prove detrimental. As Rabinowitch (1945) pointed out, because of their energy transducing function, plants are potentially very self-destructive organisms, and the ability of to resist destruction by avoiding a deleterious "overflow" of radiant energy is one of the most important attributes posessed by sun plants, which shade plants may lack. Red algae are particularly implicated in this, since their phycoerythrin is characteristically a very photolabile pigment both in vivo and in vitro. Accounts of sun-shade adaptation in higher plants are given by Rabinowitch (1945, 1951, 1956), Gabrielsen (1948), Bjorkmann & Holmgren (1963) and in papers presented in Bainbridge et al.(1966) and Evans et al.(1975). Sun-shade adaptation occurs in marine phytoplankton in situations where stratification of the water column maintains populations at one depth for prolonged periods (Yentsch 1962, 1963). Laboratory experiments investigating the extent of sun-shade adaptation in marine macroalgae in response to depth of growth have been carried out by Montfort (1929), Stocker & Holdheide (1938), Levring (1947), Jupp (1972) and Mathieson & Norall (1975).

Early attempts to measure the influence of the attenuation of irradiance in the sea upon photosynthesis in situ were carried out by Gaarder & Gran (1927) and Marshall & Orr (1928). Their techniques involved measuring changes in

oxygen content produced by phytoplankton samples contained in bottles suspended at different depths. Gail (1922) used a similar technique to measure photosynthesis of macroalgae collected from the shore, or from the sublittoral zone by dredging. His work was followed by the studies of Tschudy (1934), Printz (1939) and Levring (1947, 1968, 1969), all utilising material obtained by dredging.

In the early 1950's, two new techniques were developed; free diving using SCUBA (self-contained underwater breathing apparatus) and the ^{14}C isotope tracer technique for the measurement of photosynthesis, and these two methods were soon pressed into the service of marine botany. In 1952, Steemann - Nielsen (1952a) developed a ^{14}C technique for the measurement of phytoplankton photosynthesis, still using the basic incubation methods of Gaarder & Gran (1927). Although Kitching (1941) had used a surface-supply diving helmet to observe a sublittoral laminarian community, the first use of SCUBA in British waters in a wholly macroalgal study was by Kain (1960) at the Isle of Man. The direct nature of this underwater technique had a great advantage over dredge sampling of sublittoral communities. The first use of the ^{14}C method in combination with free diving was in <u>in situ</u> experiments on calcareous algae and corals, by Goreau (1963), and the method has since been used by Drew (1966) and Johnston (1969) in studies of marine macroalgae.

Studies of photosynthesis and growth in the sea have been carried out largely in relation to the contribution made by the different algal groups to marine productivity as a whole. Thus, much work has been done on the phytoplankton, by Steemann- Nielsen (1952a, 1974), Steemann - Nielsen & Jensen (1957), Ryther (1954, 1956a,b,c), Ryther & Menzel (1965), Ryther & Vaccaro (1954), Yentsch (1962, 1963), Yentsch & Lee (1966). Due to the dominance of the Laminariales in the sublittoral zone in temperate waters, much work has centred round these;<u>Macrocystis</u> spp. in the USA (see

treatise by North 1971) and in Europe, on Laminaria spp. (Walker & Richardson 1956; Bellamy & Whittick 1968; Kain 1965, 1966, 1971; Svendsen & Kain 1971; Lüning 1971; Robertson 1970; Jupp 1972; Kain et al. 1975; Drew & Jupp 1974; Drew et al. 1976) and Saccorhiza polyschides (Norton 1969; Norton & Burrows 1969). However, field and ecological studies of the algae of the underflora have been less frequent, although pertinent information was included in papers by Bellamy & Whittick (1968), Jupp (1972), Aleem (1973) and Kain (1976). In the Mediterranean, quantitative studies of sublittoral algal communities have been undertaken by Crossett et al. 1965, Crossett & Larkum (1966), Larkum et al. (1967), Drew (1969) and Zavodnik (1971, 1973).

It was the aim of the present study to extend the knowledge of the physiological ecology of the smaller algae of Britain and the Mediterranean by utilising up to date techniques in situ and in the laboratory. The study concentrated principally on red algal species due to their unique pigment constitution, and the claims made for it by the chromatic adaptation theory. "Control" studies were made on certain green algae having the chlorophyll-based pigment constitution representative of the vast majority of the members of the plant kingdom. Modifications of the ^{14}C and oxygen methods were developed for use in short-term in situ experiments to depths of 60m in the sea. The in situ method comprised two distinct types of experiment. Firstly, the "true" in situ experiment in which algae were incubated at their site of normal growth, involving minimum disturbance, in an attempt to measure the rates of photosynthesis normally attained by these species in nature. Secondly, algal speciments were transferred to various "unnatural" depths for incubation to investgate their degree of adaptation to their normal situation. In situ photosynthesis and respiration measurements made thus, on the basis of short-term experiments, could be used to make speculations

concerning the growth patterns of the algae studied, in relation to irradiance, time of day and season.

The SCUBA method does impose certain limitations which are not present in a "suspended bottle" technique. These include limited bottom-time at depths in excess of 30m, nitrogen narcosis and cold, both of which impair diver efficiency, time-consuming decompression stops and a susceptibility to inclement surface conditions which curtail diving operations. Set against these disadvantages are the considerable advantages of ensuring that plants selected for experiments are healthy and representative of the local flora, and that the plants are not removed from their environment and exposed at the surface as is necessary in a dredge collection method.

Since diving operations were shore-based, this to a certain extent governed the choice of experimental site. Limited studies of the hydrography and ecology of the field sites were made, to provide a background to the physiological findings. In general, algal species were chosen for study on the basis of as many of the following conditions as possible:

(1) Being typical of the flora of the area, although not necessarily dominant on a biomass basis

(2) Having a wide depth range

(3) Being typically "deep" or "shallow" species

(4) Having thalli of a form convenient to the methods used, usually flat and expanded, permitting discs to be cut

(5) Having a wide geographical range to allow inter-comparison between sites.

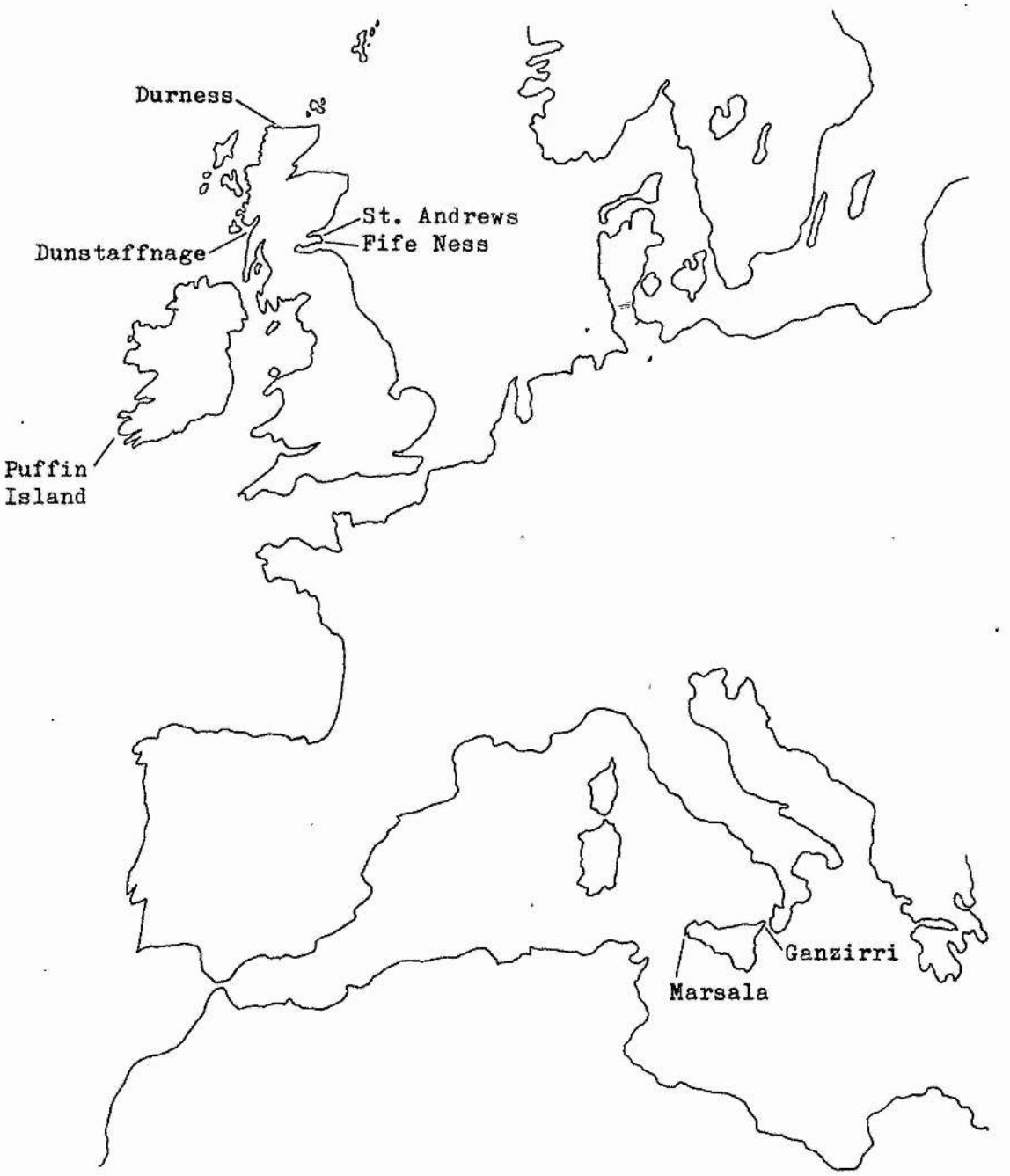


Figure 1.2. Map of Europe showing positions of experimental sites.

Measurements were made of certain physical and physiological parameters besides photosynthesis and respiration, to help assess the degree of adaptation of the algae with respect to depth of growth.

In field experiments, because experimental parameters are largely uncontrolled, these should be monitored if at all possible (see Šesták 1971, p.31) and, in addition to measuring temperature, special attention was paid to the measurement of irradiance, because of the importance of this factor in controlling photosynthesis in the sea, as discussed above. Because of the importance of the irradiance aspect, studies were also conducted in the laboratory, under controlled conditions of irradiance and temperature. In situ experiments were necessarily conducted under static or unstirred conditions, and because of the effect this might have on Blackman's (1905) "conditions of supply of material" and therefore on the measured rates of photosynthesis and respiration, an assessment was made, in the laboratory, on the effect of water movement on metabolism of algae.

Figure 1.2 shows the positions of the sites at which work was carried out in the present study. In situ experiments were conducted at all sites except St. Andrews which was used only as a source of littoral algae for use in laboratory experiments. The deepest site in the British Isles was at Puffin Island, where experiments were conducted at 18m. Due to the much more predictable and generally more clement conditions and greater water clarity prevailing in the Mediterranean, diving to a depth of 60m was feasible at Ganzirri and experiments could be conducted there at that depth on a regular basis.

In summary, then, in the present study techniques have been developed enabling the short-term measurement of photosynthesis in situ and in the laboratory. The results of such experiments have been discussed in terms of the observed communities of which the species studied were part and in relation to irradiance, one of the major controlling influences on plant

colonisation underwater. Due to the importance of water movement in methodology and in the environment, experiments and discussion on this topic have also been included. Dark respiration rates, measured in the field and the laboratory, enabled discussion to be made on the possible patterns of net growth on a twenty-four hour, and also seasonal basis.

CHAPTER 2

Materials and methods

CONTENTS

1. Introduction

Many of the techniques discussed in the present chapter involve experimentation on algae in the field and some form of subsequent analysis back at St. Andrews. The bulk of the chapter concerns the measurement of photosynthesis. There are nine basic techniques for photosynthesis measurement (Heath 1969; Šesták et al. 1971) and the two used here involve measurement of (1) accumulation of photosynthetic products - ^{14}C method and (2) oxygen efflux - oxygen method. The ^{14}C method requires a relatively small number of operations to be carried out in the field, which has the advantage that a relatively large number of experimental treatments can be carried out and the material subsequently stored for several months before analysis. The oxygen method must be completely carried out in the field (usually in a field laboratory) which, although time-consuming, has the advantage of "feed-back" of results allowing continual re-assessment and modification of experimental design. In both techniques, in experiments conducted in the laboratory at St. Andrews, analyses of the plant material normally proceeded immediately after the termination of the experiment.

2. Plant material for experiments.

a. Collection for experiments in the field

For *in situ* experiments, algae were collected at the experimental site and used immediately. For "transfer" experiments, algae were brought to the surface and transported in black polythene bags for incubation at other depths or at the surface.

b. Collection for laboratory experiments at St. Andrews

Littoral species were collected from intertidal rocks and transported in sea water to the laboratory 500m distant. They were either used in experiments immediately or kept in the laboratory aquarium under conditions of low irradiance (0.5m W cm^{-2}) until required (never more than 48 hours).

Sublittoral species were collected by divers at Fife Ness and transported from their site of growth to the laboratory in sea water in the dark.

c. Species investigated

Samples of the species used in experiments were collected and preserved for later identification either as pressed herbarium specimens or in 4% formalin in seawater. Identifications were verified in most cases by Mr. J.H. Price, British Museum (Natural History). The species which were subjects of physiological experiments are listed in Table 2.1. Basically the nomenclature follows that of Dixon (1973) in that the major

groups have been treated as Divisions (Rhodophyta, Chlorophyta) and the two major subdivisions of the Rhodophyta were therefore considered as classes (Bangiophyceae, Florideophyceae). Species nomenclature follows the Check List of British Marine Algae - Second Revision (Parke & Dixon 1968) and for species not included in that publication the nomenclature is that in Fauna and Flora der Adria (Riedl 1963). A note on the taxonomic status of Ulva specimens used in the present work appears on p.151.

Table 2.1. List of species used in physiological experiments.

* Species studied at Ganzirri only

** Species studied at Ganzirri and British Sites

Without asterisks, species studied only in Britain

RHODOPHYTA

Bangiophyceae

Bangiales

Bangiaceae

Porphyra leucosticta Thur. in Le Jol

**Porphyra umbilicalis (L.) J.Ag.

Florideophyceae

Nemaliales

Bonnemaisoniaceae

Bonnemaisonia asparagoides (Woodw.) C. Agardh

Gelidiaceae

*Pterocladia capillacea (Gmel.) Born. et Thur.

Cryptonemiales

Dumontiaceae

Dilsea carnosa (Schmidel) Kuntze

Dumontia incrassata (O.F. Mull.) Lamour.

Squamariaceae

**Peyssonelia* sp. [a]

Corallinaceae

**Pseudolithophyllum expansum* (Phil.) Lemoine

Kallymeniaceae

Callophyllis laciniata (Huds.) Kutz.

Kallymenia reniformis (Turn.) J.Ag.

Gigartinales

Gracilariaceae

**Gracilaria verrucosa* (Huds.) Papenf.

Plocamiaceae

Plocamium cartilagineum (L.) Dixon

Sphaerococcaceae

**Sphaerococcus coronopofolius* (Good et Wood) C.Ag.

Rhodymeniales

Rhodymeniaceae

Rhodymenia palmata (L.) Grev.

Ceramiales

Rhodomelaceae

**Laurencia obtusa* (Huds.) Lamour

Laurencia pinnatifida (Huds.) Lamour

Odonthalia dentata (L.) Lyngb.

Polysiphonia lanosa (L.) Tandy

**Vidalia volubilis* (L.) J. Ag.

Delesseriaceae

Delesseria sanguinea (Huds.) Lamour

Nitophyllum punctatum (Stackh.) Grev.

Phycodrys rubens (L.) Batt.

Polyneura hilliae (Grev.) Kylin

CHLOROPHYTA

Ulotricales

Ulvaceae

Enteromorpha linza (L.) J.Ag.

**Ulva lactuca L.

In the text of this thesis, for the sake of simplicity, algal species studied will be referred to by their generic name along, where this does not result in any ambiguity. In only two genera were more than one species used in physiological experiments. These were:

(1) Porphyra umbilicalis and P.leucosticta, the latter being studied in only a few experiments at Puffin Island,

(2) Laurencia pinnatifida and L.obtusa, of which the former occurred only at British sites, the latter, only in the Mediterranean.

a This is the spelling given in Parke & Dixon (1968). In the third revision of the check-list (Parke & Dixon 1976) the spelling is "Peyssonnelia". Since the genus was named after Peyssonnel, the French naturalist, it would appear that the spelling used by Parker & Dixon (1968) was incorrect.

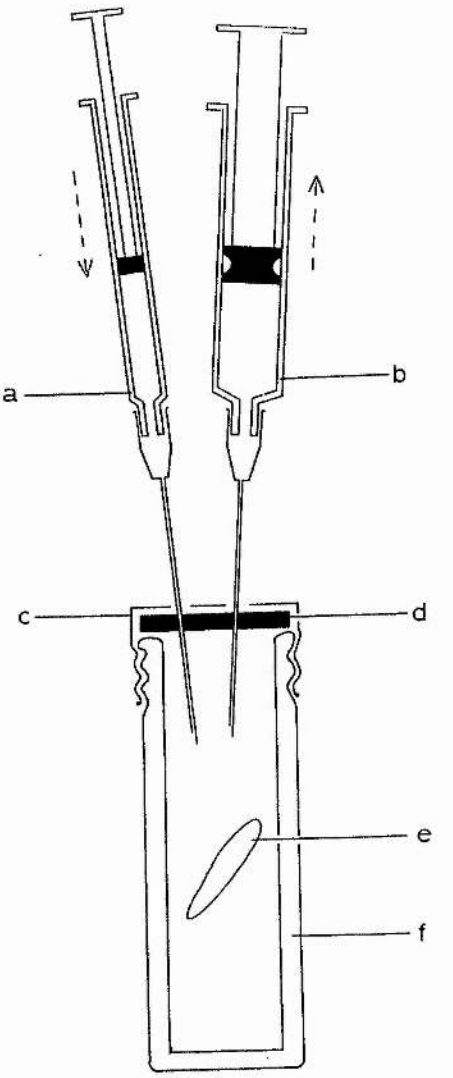


Figure 2.1. Injection procedure used in Winkler or ^{14}C techniques, a, 1ml plastic hypodermic syringe, with needle, containing Winkler reagent I, II or III, or ^{14}C radioisotope solution; b, 5 or 10ml pressure relief syringe, with needle; c, aluminium or plastic screw cap with injection ports; d, sealing rubber lid-liner; e, algal tissue disk; f, 28ml incubation bottle.

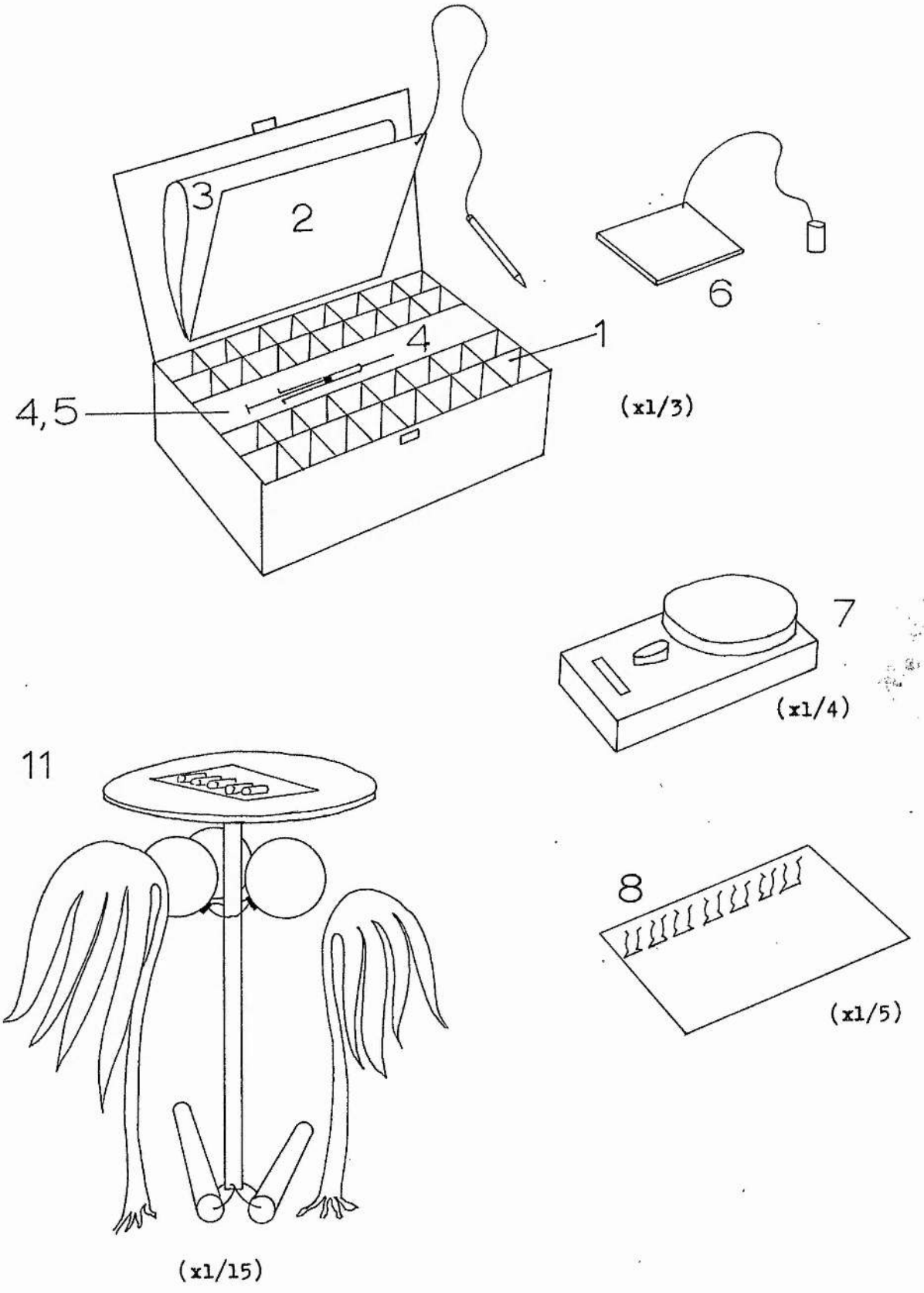


Figure 2.2. Equipment used in in situ experiments (see facing page).

3. Incubation methods

a. The incubation vessel

It was required that the vessel be sufficiently small to enable a diver to transport up to thirty-two of them to the experimental sites but also large enough to minimise the occurrence of nutrient depletion during the incubation period. The vessel used was the 28ml nominal capacity "universal container", manufactured by United Glass Limited, hereafter referred to as "incubation bottles" (See figure 2.1). The rubber sealing liner of the lid allowed leak-proof injection of radio-isotope or Winkler reagents through two small holes punched 0.75cm apart in the aluminium lids (as supplied with the bottles) or plastic lids (used latterly, supplied by Sterilin Limited). The injection procedure is illustrated by Figure 2.1. In the Winkler oxygen method, reagent injections were 0.5ml in volume, in the ^{14}C method, radio-isotope injections were 0.2ml in volume. The relief syringe was necessary to allow injected fluid to displace an equal volume of seawater, and was sufficiently large to last for at least ten injections. Only ^{14}C radio-isotope injections were carried out underwater.

b. In situ method

(i) Apparatus and equipment

Most of the apparatus required in an in situ experiment was contained in a portable galvanised steel box (Figure 2.2) with hinged lid and internal compartments, containing the following items:

1. Up to thirty-two incubation bottles with lids
2. Formica writing-board with attached pencil
3. Black polythene bags for enclosing "dark" incubation bottles, and for collection of experimental material for transport to other sites
4. Plastic disposable syringes of 1ml capacity, fitted with needles and containing isotope solution
5. 10ml plastic relief syringe fitted with needle
6. Weighted plywood block with attached disc-cutter of 2.26cm diameter brass tube sharpened to cut discs of tissue 4 cm^2 in area.

Also carried by the diver, in a plastic mesh bag

7. Submersible integrating radiometer (see p. 43)
8. Platforms of galvanised steel fitted with "Terry" spring clips to hold bottles in place during incubation.

Worn on the diver's wrist :

9. Depth guage, Bourdon-tube pattern
10. Diver's watch.

In Britain, where Laminaria hyperborea usually dominates the shallow sublittoral zone, shallow (around 3m depth) incubations were carried out on buoyant platforms which projected above the L.hyperborea canopy, (Figure 2.2, no. 11) as used by Jupp (1972) and Drew (1973a). Similar platforms to those used at the deep sites (no. 8) could be clipped to and unclipped from, the large buoyant platform.

(ii) Procedure

Experiments were usually timed to commence before, and terminate after, noon so that a fairly high and relatively invariable irradiance obtained during each incubation. The equipment box was prepared beforehand with marked bottles, Formica board with full experimental details, and the required number of syringes filled with the prepared bicarbonate radio-isotope solution.

Most experiments involved only two underwater stations, one shallow, from 0-4.5m depth, and one deep, from 12-60m depth. Also, most experiments involved the incubation of "shallow" plant material simultaneously at shallow (i.e. in situ) and deep (i.e transferred) stations, and vice versa for "deep" material. Thus, the procedure commenced with a short dive at the shallow station to collect experimental material in black polythene bags, these were placed in the equipment box and transported to the surface above the deep site by a short boat journey, and the incubation bottles were filled with water to prevent the implosion of the rubber cap liners at depth. A dive was then made to the deep site, the equipment box opened and a collection of deep-growing algal material was made. Discs were then cut from selected plants of both deep and shallow material (in branched forms, fragments of the plants were used) and placed in the pre-marked bottles in accordance with the instructions on the Formica board. The lids were then replaced on the bottles which were then returned to the box to await injection, in the ^{14}C method. Care was taken when placing the tissue in the bottles, to flush out the bottles with a finger to ensure that water from the deep site, and not surface water, bathed the tissues. When all bottles were complete with tissue, each was injected with 0.2 ml of the isotope solution, as described

above (p.19) and clipped into place on the incubation platform which was itself placed (at deep sites) on a clear substrate, without any shading by other algae. Bottles involved in the oxygen method were clipped onto the platforms without further manipulations. For dark, incubation, the bottles involved were placed in black polythene bags. At this point the integrating radiometer was placed beside the platform, the reading noted on the Formica board and the instrument switched on. The time, temperature and the depth of the platform were then noted. Deep material for incubation at the shallow site was then collected in black polythene bags and placed, with the other equipment, in the metal box. At this point the diver returned to the boat, carrying out decompression procedures if necessary, and revisited the shallow site, usually about one hour from the commencement of the whole operation. A similar procedure was then carried out at the shallow station, this time placing the bottles in the small platform, itself clipped to the buoyant platform floating 1.5m above the sea floor. The incubation period lasted from 1-4h in Britain and 4-8h at Ganzirri. After this the experiment was retrieved by a diver who collected the platforms in black polythene bags, again noting the integrating radiometer reading, time, temperature and depth, at each site. The time which then elapsed between retrieval from the sea floor and the effective termination of the incubations in the laboratory ashore, were noted and taken into account in subsequent calculations. Termination of ^{14}C experiments is described in detail on p. 29 and of oxygen experiments on p. 37.

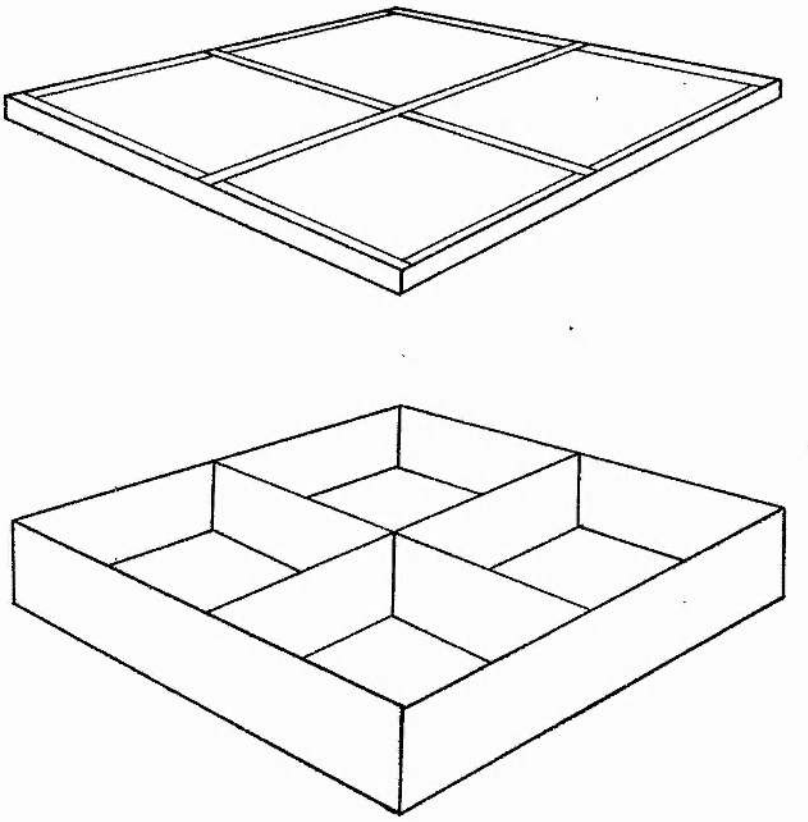


Figure 2.3. Incubation chamber used at the surface, in the field, with 3mm thick clear Perspex lid holding neutral density filters.

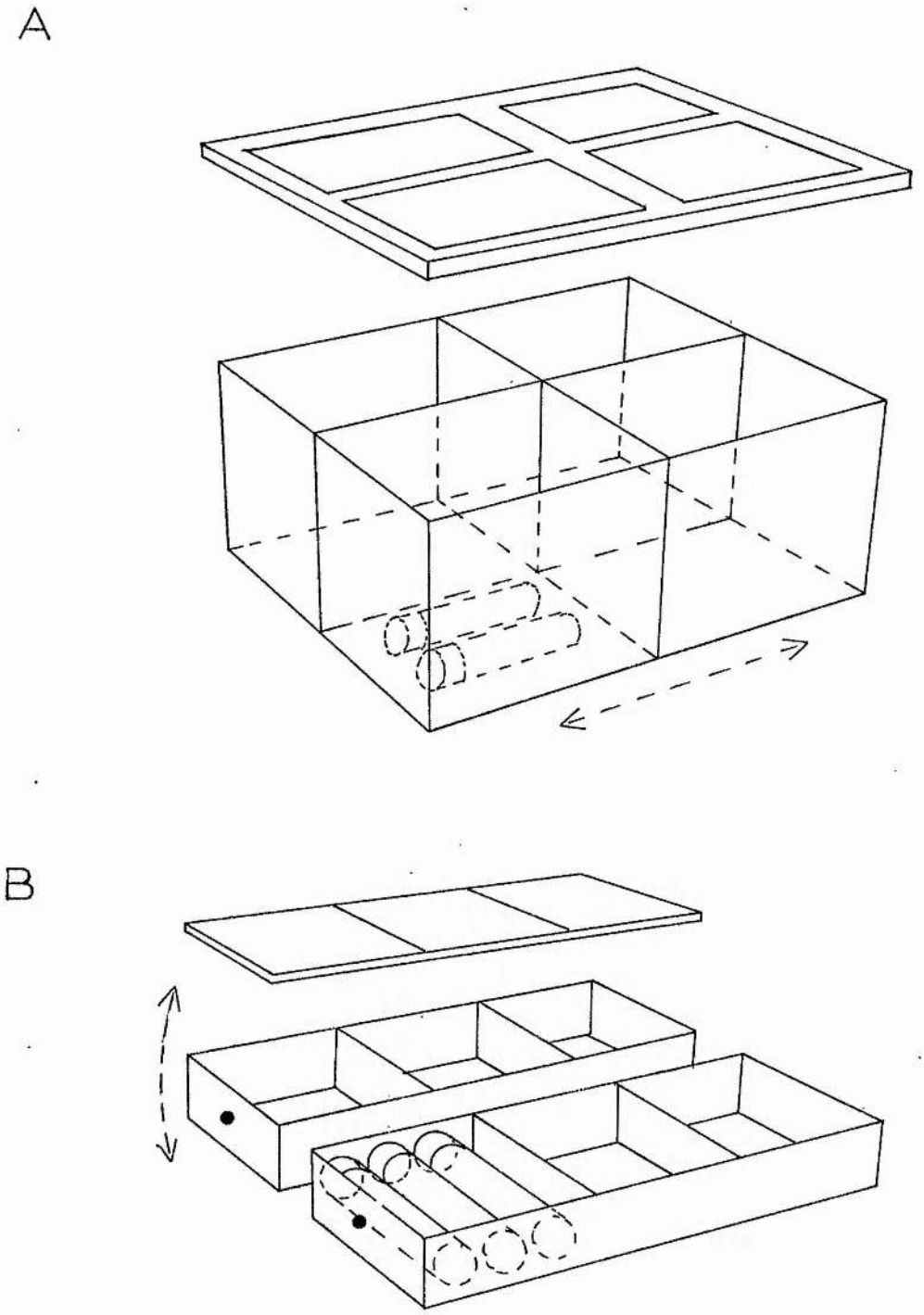


Figure 2.4. Laboratory incubation chambers used with tungsten-iodide light source; A, black Perspex chamber with lid holding neutral density filters; B, two tilting trays, each with lid holding neutral density filters. Arrows show direction of shaking movement.

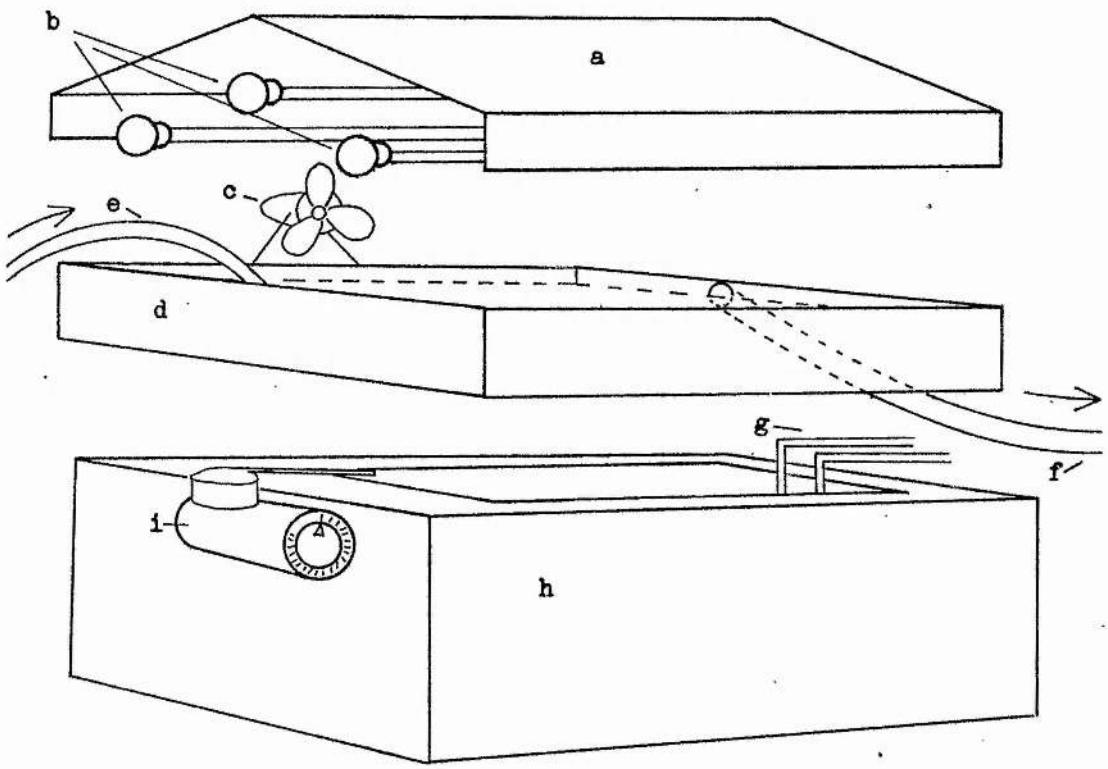


Figure 2.5. Constant temperature shaker bath used in laboratory photosynthesis experiments; a, reflector; b, 3 tungsten-iodide lamps; c, cooling fan; d, infrared water filter; e, inflow; f, outflow; g, supply to cooling coils; h, bath unit with thermostated heater; i, shaker motor assembly.

c. Surface incubation

For field incubation of material irradiated by natural daylight above the surface of the sea, a simple incubation chamber was constructed (Figure 2.3) of steel (painted white inside) with four compartments and a close-fitting lid of 3mm thick clear Perspex. Different numbers of sheets of Cinemoid No. 60 (Pale Grey) neutral density film could be taped over the box compartments to produce four levels of irradiance. Each compartment held four incubation bottles and the chamber was used full of seawater, submersed to a depth of approximately 1cm in a tank of seawater to ensure constant temperature. Such incubations could be regarded as being in situ for shallow growing species such as Porphyra, Rhodymenia, Laurencia, etc.

d. Laboratory incubation

Fragments of branched species or discs of laminar species were prepared in plastic tanks of seawater in the isotope laboratory. Incubation bottles were filled by submergence in a deep tank of seawater (drawn directly from the main laboratory aquarium), experimental tissue was introduced into the bottles and their caps screwed on underwater. At this point, in the ^{14}C method, prepared radio-isotope solution was injected into the incubation bottles. The bottles were incubated in a four-compartment light-tight incubation chamber of black Perspex (Figure 2.4A) set in the oscillating carriage of a Gallenkamp constant temperature shaker bath (Figure 2.5). The water bath was provided with a thermostated heater which, together with separate cooling coils in both chamber and bath, fed by an external refrigeration unit, provided a temperature range of 8-30°C. For incubation at various levels of irradiance, the chamber lid was fitted with a recess above each compartment, capable

of holding one of three Kodak Wratten neutral density filters producing attenuation to 31.5%, 10% and 1% of incident irradiance. Irradiance could be further varied by varying the number of tungsten-iodide lamps (up to three) used, see p. 45. Dark incubations for dark ^{14}C fixation measurement, or dark respiration measurement, using oxygen technique, could be achieved either by occluding the filter carrier of one compartment with a close-fitting square of black perspex sheet in place of a neutral density filter, or by wrapping the incubation bottles in either aluminium foil or black polythene. When a series of irradiance levels was not required, the incubation chamber could be removed, and bottles clipped directly to the floor of the oscillating carriage of the constant temperature bath.

The system was latterly modified to allow a different shaking system to be operated. Bottles were incubated in two parallel trays which could be tilted about a central axis (Figure 2.4B). Each tray had three shallow compartments holding three incubation bottles and fitted with a lid containing a number of Cinemoid No. 60 neutral density filters producing attenuation to 30%, 9%, 2.7%, 0.8% and 0.24% of ambient irradiance.

Further details of both modes of shaking are given in Chapter 3.

e. Incubation in flowing water (see Figure 2.6)

This system was designed specifically for a series of experiments described in Chapter 3, and was used in no other experiments. A peristaltic pump delivered sea water via manifolds, to four Perspex sample tubes, each 30cm long with internal diameter 2.5cm. Each contained a Perspex sample carrier, fitted with stainless steel pins for mounting the plant tissue. Taps fitted at either end of one tube enabled this to be used as a

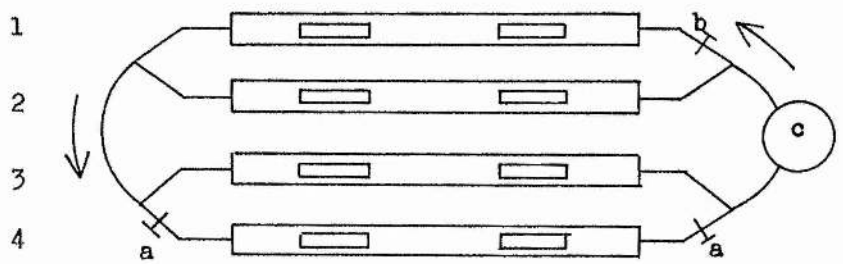


Figure 2.6. Schematic diagram of flowing water incubation apparatus showing 4 Perspex tubes containing tissue samples; a, taps closing off flow to tube 4; b, clip on inlet to tube 1, controlling flow rate; c, peristaltic pump. In order of magnitude, flow rates are 3, 2, 1, 4.

control in which there was no water flow. By manipulating a screw clip on another of the inlet manifolds, the water flow rate could be made different in the remaining three tubes. Flow rate was measured in each tube while the experiment was in progress, by timing the movement of minute particles of suspended matter present in the sea water, along a measured ten centimetre portion near the middle of each tube. Maximum flow velocity was 5cm s^{-1}. There was found to be a turbulent zone extending 10cm from the inlet manifold in each tube; plant tissue was therefore always placed further than 10cm from the inlet. Particles were timed only when travelling down the centre of the lumen of the tubes, since this was where the tissue was situated, and also where flow rate could be expected to be maximal. The flow of tap water through the surrounding water bath maintained a constant temperature of 13°C. Irradiance was supplied by an array of six fluorescent tubes (see p. 45).

A criticism of the apparatus was that the high frequency ($\sim$2 cycles s^{-1}) pulsating nature of the flow induced by the three-roller pump may have produced local motion of water, close to the plant surface, of a higher velocity than that measured over longer distances. Such high frequency water movement has been noted by Munk & Riley (1952) to cause enhanced uptake of external solutes by phytoplankton. Also, the higher velocity of water flow recorded in the centre of the lumen was similar to the "boring" effect described by Westlake (1967) in a similar apparatus, but since the plant tissue was placed in the centre of the lumen only, it can be assumed that the rate of flow around the tissue was relatively homogeneous. In any subsequent development of this technique, however, it is recommended that a non-pulsating peristaltic pump, or an impeller-type pump be used, and baffles provided at theinlet and outlet manifolds to prevent boring.

Since the tissue was not perfectly flat and parallel to the direction of flow, it is probable that flow over its surface was not perfectly laminar. However, this would also be the case in the natural environment, and the apparatus can be assumed to replicate relatively closely a natural situation in which an attached plant was growing in a low velocity current.

4. ^{14}C Technique

a. Introduction

The first use of this technique in the marine environment was by Steeman - Nielsen (1952a) who used it to measure phytoplankton photosynthesis. It has subsequently been adapted for use with marine macroalgae and the present method was based on that developed by Drew (1966), Drew & Larkum (1967), Drew (1969), Drew (1973a & b), Drew & Jupp (1976) and Drew et al. (1976). The principle of the technique is described by Heath (1969) and Šesták et al. (1971) and involves the addition of ^{14}C to the normal ^{12}C source pool utilised by the plant, incubating the plant material in this mixture in the light, subsequently killing the tissue and measuring the amount of radioactivity therein. From a knowledge of the specific radioactivity of the total inorganic carbon in the bathing solution (units of radioactivity per unit carbon) the amount of total carbon fixed, represented by the radioactivity in the sample, can be calculated.

In seawater, carbon dioxide exists in the form of a buffer system, represented by the equation,

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^{--}$$

the equilibrium shifting towards the right with increase in pH. At pH≒7-8 which is normal for seawater, approximately 90% of the inorganic carbon is present as bicarbonate ion (HCO_3^-), and carbon in this form is utilised by many, if not all, marine macroalgal species (see p. 93). Thus in the present ^{14}C method, the isotope was added in the form of $NaH^{14}CO_3$ solution made up in seawater.

As in terrestrial plants and phytoplankton, marine macroalgae fix carbon into organic compounds in the dark to a small extent, and it is a premise of the ^{14}C method that, being independent of photosynthesis, this process continues unchanged in the light. (See also p. 62) In order to allow for this non-photosynthetic fixation of carbon during incubation with ^{14}C in the ^{14}C method, dark control incubations were always carried out concurrently with the light incubations. In subsequent calculations, carbon fixation rate in the dark was then subtracted from carbon fixation rate in the light to produce the photosynthetic carbon fixation rate (see also p. 34).

b. Radio-isotope

The radio isotope was supplied by the Radiochemical Centre, Amersham, in sealed glass ampoules containing 1000 μCi (microcuries) of Na $H^{14}CO_3$ in 0.5 ml of solution made up in distilled water. This was diluted with sea-water to produce a stock solution of concentration 10 μCi ml^{-1}.

In all cases where the 28ml incubation bottles were used for incubation, 2 μCi of ^{14}C were injected in the form of 0.2 ml of a 10μCi ml^{-1} solution of $NaH^{14}CO_3$ in seawater. (The addition of approximately 10 μl of distilled water, from the original stock solution, with each injection, would produce a negligible decrease in salinity of the bathing seawater). Norris et al.(1955) used approximately 100 times this radio-activity in work with microalgae and higher plants and Holm-Hansen et al.(1958) treated Chlorella with one million times as much. Both detected no significant effect of radioactivity on photosynthesis. Bidwell (1974, p.572) has since suggested that high specific radioactivities of $^{14}CO_2$ could strongly influence the reactions of photosynthesis. However, it has been assumed that the extremely low levels used in the present work had no deleterious effects on the algae studied.

c. Killing and storage of tissue

(i) Direct method

This method was used only with discs of membraneous species (e.g. Porphyra, Delesseria, Ulva). The discs were removed from the incubation bottles with forceps, dipped in distilled water to remove adherent seawater, blotted dry with paper tissues and placed on 3cm diameter aluminium planchets. These were immediately transferred to an oven at 100°C (a Calor-gas oven in the field) left until dry and then stored for transport back to St. Andrews in specially made Perspex trays with close-fitting lids. The water-filled incubation bottles were retained and also transported back to St. Andrews for radioactive assay.

(ii) Indirect method

Plant discs or fragments were removed from the incubation bottles, rinsed briefly in distilled water and transferred to further 28ml bottles containing 5-10 ml of 80% ethand which killed the algal tissue immediately. With their caps screwed on these bottles were stored in boxes for transport back to St. Andrews together with the incubating-water samples as before.

d. Preparation of tissue fractions in indirect method

These operations were all carried out in the isotope laboratory at St. Andrews.

(i) Alcohol extract

Extraction was completed by using three changes of boiling 80% ethanol; these extracts were combined and made up to 25ml standard volume.

(ii) Acid hydrolysate

The extracted tissues were dried to constant weight in foil cups in an oven at 100°C and weighed. The tissue samples were then transferred to 50ml test tubes and 5ml of 0.5M sulphuric acid added. A foil cap was put on each tube to reduce evaporation and the tubes heated at 100°C in a water bath for three hours. The tubes were then removed, allowed to cool and the acid in each tube decanted off the remaining algal tissue into a fresh 28ml bottle. The tissue was washed three times with distilled water, the washings added to the acid and made up to 25ml standard volume.

(iii) Insoluble residues

The remaining algal tissue samples were retained and soaked in a large volume of water to remove traces of acid.

e. Preparation of samples for radioactive assay

(i) Direct method

Because of their hygroscopic nature, it was necessary to re-dry the unextracted tissue discs on their planchets, but a drop of glacial acetic acid was added at this stage in any case, to drive off any residual inorganic ^{14}C as $^{14}CO_2$ gas. The discs were then dried by warming the planchets, since the natural mucilages of the algae generally resulted in a close adhesion of the discs; however, water-soluble gums or double sided sellotape were used when discs came loose, although this interfered with the subsequent determination of dry weight. This method of assaying intact tissues has been successfully employed for leaves of high plants (Austin & Longden 1967; Campbell 1972).

The planchets, with their samples, were weighed prior to counting and the tissue areas measured. After counting, each tissue sample was washed from its planchet, all the planchets dried and re-weighed. The dry weight and area data were used in the computation of densities and thence, self-absorption corrections.

(ii) Indirect method

Alcohol fraction 0.1ml of each sample was applied to each of two replicate planchets, together with one drop of glacial acetic acid, again to remove residual inorganic ^{14}C. Previously, with a wax pencil, a wax ring had been drawn round the inner margin of each planchet, while warm, and this prevented the ethanol sample from creeping up the planchet sides and so upsetting the counting geometry. Again, the samples were evaporated to dryness by warming on a hotplate at 40°C

Acid hydrolysate 2ml from each sample were transferred to 5ml centrifuge tubes and neutralised with barium carbonate, testing with BDH Universal pH paper. The tubes were then centrifuged to remove the resulting insoluble barium sulphate and excess barium carbonate. 0.25ml aliquots were transferred from the clear supernatants onto separate planchets (wax ring not required for aqueous solutions) and dried on a hotplate at 40°C.

Insoluble residues The supernatant washing water was carefully poured off and discarded, and each residue was scraped from its test tube using a spatula, macerated on a weighed planchet into a thin and homogeneous layer, dried at 40°C as above and the planchet + sample re-weighed. The areas of the dried residues were measured, and used, together with the dry weights, to calculate sample densities (mg cm^{-2}) which were used in the calculation of self-absorbtion corrections.

(iii) Specific radioactivity of water samples

2ml of seawater from each of the original incubation bottles were transferrred to 10ml centrifuge tubes, and 2ml of a saturated solution of barium chloride were added. The resulting precipitate of barium carbonate (and other insoluble salts of barium) was removed from suspension by centrifugation and the supernatant discarded. The precipitates were then washed twice by re-suspension in 5ml distilled water to remove soluble

salts. Finally, each precipitate was re-suspended in 1 ml distilled water, and 0.25 ml of this suspension was applied to a weighed planchet and dried on a hotplate. The areas of the dried precipitates were measured and used together with their weights, to calculate self-absorption corrections.

f. Counting

(i) Procedure

The radioactivity of all planchet samples was counted using an automatic sample feed Nuclear Chicago "Biospan" Model 4338 gas flow proportional counter with decade scaler and printing lister. The machine used a gas mixture of 90% argon : 10% methane, designed to diffuse through the thin "Micromil" end-window and envelope the sample, giving the instrument a relatively high counting efficiency of around 20%. Each sample was counted for three periods of ten minutes each, and the count numbers were averaged and count rates expressed as counts per minute (cpm).

(ii) Corrections to the count rates

Background counts Background radiation was measured using one clean planchet in each series of samples. Counts per minute due to background radiation (from 10-17 cpm) were subtracted from all sample counts.

Self-absorption In planchet samples of density exceeding approximately 0.5 mg cm^{-2}, beta particles originating at the distal side of the sample will be absorbed by intervening material before they can enter the counting chamber. This error was allowed for by correcting to "infinite thinness" (i.e. 0mg cm^{-2}) using self-absorption data for elements of medium atomic weight (see nomogram, Wilson 1966, p. 265).

Table 2.2 shows some values used to correct for self-absorption in the present work.

Table 2.2 Correction for self-absorption

Sample density mg cm^{-2}	% of total counts recorded
1	88
5	59
10	40
15	27
20	20

Dead time The maximum counting rates encountered were approximately 3000 cpm (i.e. one count per 20 milliseconds) and thus not sufficiently high to require correction due to the inherent dead time of the machine, which was 6 microseconds.

g. Calculation of carbon fixation rate

The specific radioactivities of the inorganic carbon in the incubation water samples were calculated by dividing the cpm per ml of water by the number of micrograms of carbon per ml, giving a value in units of cpm μg^{-1} C.

In the direct method, where algal tissue was counted intact,

$$\text{carbon fixation} = \frac{t}{s}$$

where t = total tissue cpm

s = specific radioactivity of incubation water.

This value was then divided by the incubation time (in hours) and original tissue area (in cm^2) to give a carbon fixation rate in $\mu gC\ cm^{-2}\ h^{-1}$

In the indirect method, the alcohol extract and acid hydrolysate cpm of planchet samples were first calculated for the whole 25 ml standard volume of each. Then,

$$\text{carbon fixation} = \frac{a + i + r}{S}$$

where, a = total alcohol soluble cpm
i = total acid hydrolysate cmp
r = insoluble residue cpm
S = specific radioactivity incubation water.

Again, the resulting value was divided by the incubation time and tissue area to give a carbin fixation rate in μg C cm^{-2} h^{-1}.

In both methods, the rate of carbon fixation in the dark was then subtracted from the rate of carbon fixation in the light to give the photosynthetic carbon fixation rate, in units of μgC cm^{-2} h^{-1}

In either method, rates could equally well be calculated in terms of dry weight, as μgC mg^{-1} h^{-1}.

Table 2.3 A and B shows specimen calculations of carbon fixation rate using direct and indirect methods, respectively.

Table 2.3 Calculation of carbon fixation rate using ^{14}C method

A. Direct method : Duration 3.83 hours, Tissue area 4 cm^2

Treatment	Corrected tissue cpm(t)	Specific radioactivity of seawater (S) cpm μgC^{-1}	carbon fixation μg C	carbon fixation rate $\mu gC\ cm^{-2}h^{-1}$	Photosynthetic carbon fixation rate $\mu gC\ cm^{-2}h^{-1}$
Light	17142	399	42.9	2.80	2.59
Dark	662	210	3.2	0.21	-

B. Indirect method : Duration 3.67 hours, Tissue area 8 cm^2

Treatment	Total Alcohol cpm (a)	Total acid hydrolysate cpm(i)	Insoluble residue cpm(r)	Total cpm	(S) $cpm\mu gC^{-1}$	carbon fixation μgC	carbon fixation rate $\mu gC cm^{-2}h^{-1}$	Photosynthetic carbon fixation rate $\mu gC\ cm^{-2}h^{-1}$
Light	31688	42010	3917	77615	287	270.4	9.21	9.18
Dark	313	123	26	462	456	1.0	0.03	-

5. Winkler oxygen technique

a. Introduction

A method was devised by Winkler in 1888, to determine concentrations of dissolved oxygen in aqueous solutions, and this method has since undergone many modifications, modern versions of which have been critically reviewed by Carritt & Carpenter (1966) and Phillips (1973). Its potential as a method of monitoring change in oxygen concentration due to photosynthesis and respiration of marine algae *in situ* was first realised by Gaarder & Gran (1927), working with phytoplankton. These workers instituted the now widely used "dark/light bottle" technique. The system involves three separate treatments, *viz.*, "light bottles" containing seawater and algal specimens exposed to light, "dark bottles" containing seawater and algal specimens but kept in complete darkness, and "control bottles" containing only seawater. Relative to the control bottle, oxygen concentration is expected to rise in light bottles if there is net photosynthesis, and fall in dark bottles due to dark respiration.

The modification of the oxygen technique used in the present work has been comprehensively described by Drew & Robertson (1974a and Appendix 1) but a brief outline is presented below.

b. Method

The essence of the Winkler oxygen technique is that dissolved oxygen is "fixed" or converted, by a series of chemical reactions, to a chemically equivalent amount of aqueous iodine, which can be assayed by titration with standard sodium thiosulphate solution. Unlike the ^{14}C method, in which samples can be kept for relatively long periods of time

before assay, the initial operations of the oxygen method require to be carried out immediately, although later stages of the process can be executed up to 48 h after termination of the experiment. On arrival at the field laboratory, incubation bottles were removed singly from the black polythene bags, in dim light, then, without removing the lids, reagents I (manganous sulphate) and II (alkaline potassium iodide) were injected and the bottles shaken vigorously. This procedure "fixes" all dissolved oxygen in the water sample as a precipitate, and, due to the high pH (about pH 14) produced in the incubation bottle by reagent II, the plant tissue was quickly killed. (Blinks, 1963, found that all of twenty four macroalgal species studied ceased metabolic activity at pH 10). At this stage, the bottles could be left in the dark for up to 48 h without change in the resultant titre. (See appendix 1, Figure 1) Before titration, reagent III (sulphuric acid) was injected, dissolving the precipitate. This operation should be done immediately prior to titration, to avoid long exposure of plant material to low pH ($\sim$2), which may cause hydrolysis and consequent weight loss. The resulting golden coloured iodine solution was decanted from the bottle (leaving the tissue behind), into a 50 ml conical flask, and titrated with reagent IV (0.05 M sodium thiosulphate solution, contained in a 1 ml hypodermic syringe with needle) using six drops of 1% starch solution as indicator. The titre of sodium thiosulphatewas equivalent to the original oxygen content of the water sample. The plant tissue was removed from the bottle, rinsed in distilled water to remove sulphuric acid, and placed on numbered squares of aluminium foil in an oven at 100^{o}C, to dry. the dried tissues were carefully wrapped in their respective foil squares for transport back to the St. Andrews laboratory where dry weights were determined.

c. Calculation

The oxygen content of the water contained in each bottle was calculated from the thiosulphate titre using the formula:

$$\text{Oxygen content} = \frac{280 \times T \times V}{V - 1.5} \quad \mu l\ O_2$$

where, 1 ml of 0.05 M sodium thiosulphate = 280 $\mu l\ O_2$

T = Titre of sodium thiosulphate (ml)

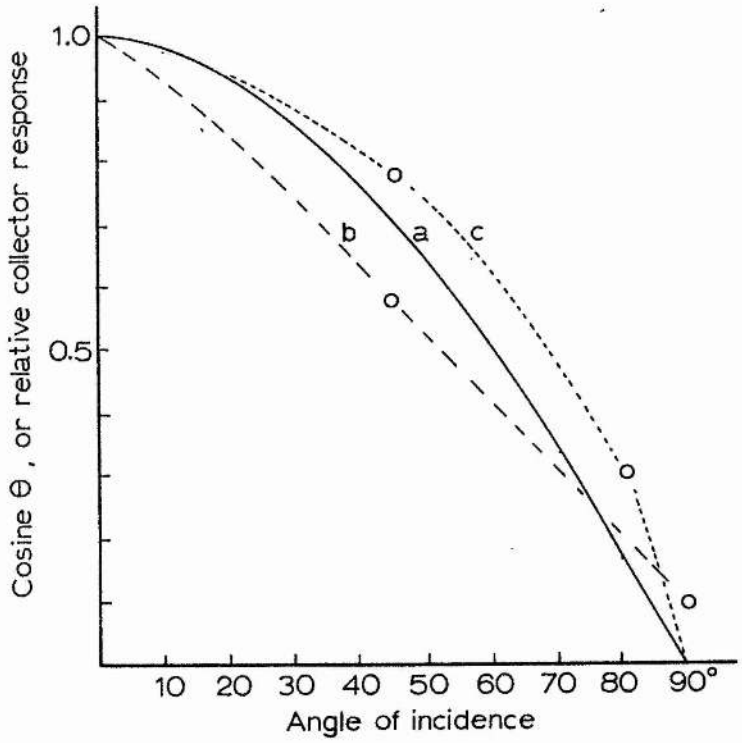
V = exact volume of bottle (ml)

and 1.5 ml of original seawater content was displaced by addition of reagents I, II, III.

A sample calculation of photosynthetic and respiration rates is shown in Table 2.4.

Table 2.4 Calculation of photosynthetic and respiration rates using oxygen method (experimental duration two hours)

Treatment	Titre ml	Final Bottle Oxygen content $\mu l\ O_2$	Bottle volume ml	$\mu l\ ml^{-1}$	Original Bottle oxygen content $\mu l\ O_2$	Change in oxygen content $\mu l\ O_2$	Tissue dry wt mg	Photosynthesis or Respiration $\mu l\ O_2\ mg^{-1}\ h^{-1}$
Light	1.245	349	28.5	-	167	+182	16	+5.68
Dark	0.530	148	29.0	-	170	- 22	12	-0.92
Control	0.585	164	28.0	5.85	164	0		



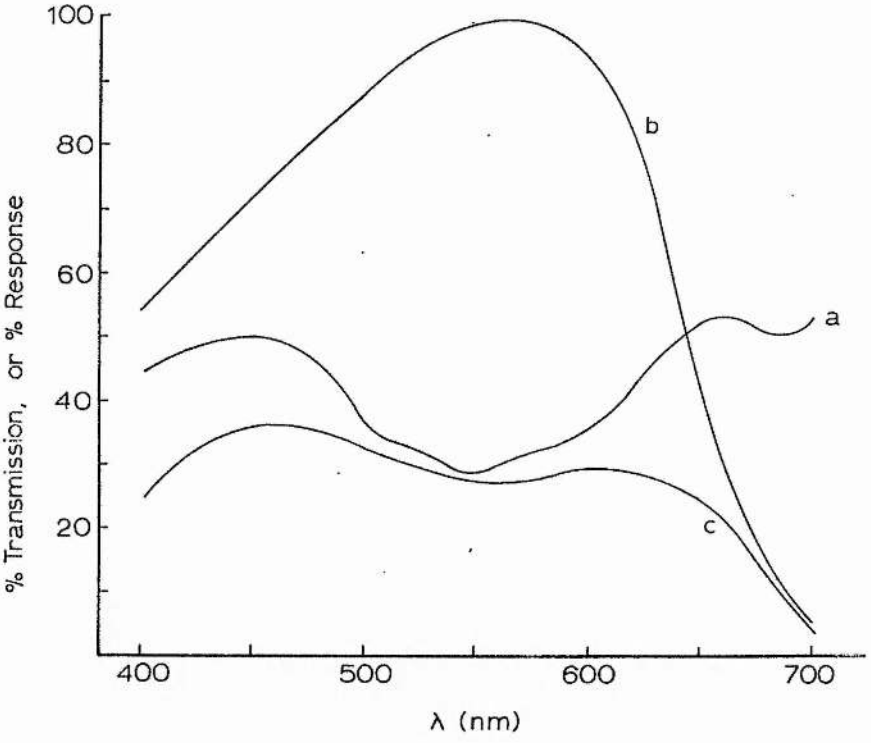


Figure 2.7. (upper). Radiometer collector responses v. angle (θ^{o}) of incident radiation; a, cos θ v. θ^{o}; b, Lintronic solarimeter; c, selenium photocell radiometer.

Figure 2.8. (lower). Spectral characteristics of selenium photocell radiometer; a, combined absorption spectrum of Cinemoid No. 9 and No. 17 filters; b, normal response of selenium photovoltaic cell; c, resulting instrument response.

6. Measurement of irradiance

a. Introduction

Three instruments were used, a commercially available thermopile radiometer with both instantaneous reading and integrating facilities, and two specially constructed submersible selenium cell radiometers, one giving instantaneous readings, the other, integrated readings.

There are two principle ways of collecting incident irradiance for measurement, one is to collect all the irradiance received by a point in space, and the other is to collect all the irradiance received by a flat plane. The instruments used were of the latter type. At an angle of incidence, θ°, of zero, (i.e. when the source is vertically above the collecting surface) the collector receives maximum irradiance, which is registered as 100% deflection by the measuring device. At an angle of incidence of 90°, no radiation is received and the deflection is zero. Between these angles, the response of the flat plane, obeying Lambert's cosine law (Monteith 1973, p.20) is in direct relation to the cosine of the angle of the incident radiation ($\cos 0^{\circ}=1$, $\cos 90^{\circ}=0$). Such a collector is said to have a "cosine" or "cosine corrected" response, shown as a plot of $\cos \theta$ against θ° in Figure 2.7 curve a.

In the descriptions below, instantaneous irradiance measurements have been expressed in units of mW cm^{-2}, while integrated measurements have been expressed in J cm^{-2}, as explained on p. 55.

b. Lintronic solarimeter

This radiometer was designed by Monteith (1959), is briefly described by Šesták et al. (1971, p. 421) and is now manufactured by Lintronic Limited, London. The device used consisted of remote detector connected by 4m of low voltage cable to a combined millivoltmeter and digital millivolt-time integrator. The detector was an eight-junction thermopile element mounted beneath the collector which was an internally ground glass dome with a specified cosine response of + 4%. However, when measured in a dark-room using the collimated light beam produced by a slide projector, the response was found to deviate from a true cosine relationship by up to 10% at 45° angle of incidence (Figure 2.7, curve b). The detector has an equal response to energy of all wavelengths in the solar spectrum between 300 and 3000 nm and thus records both "visible" or "photosynthetically active radiation" (PAR), and infrared (IR).

When used in the instantaneous mode, a meter deflection of 0.346 mV was equivalent to 1 mW cm^{-2} total energy. This mode was of use for instance, to confirm that surface irradiance was constant while underwater measurements of irradiance were in progress, and also in a study of the relative spectral composition of surface irradiance.

In the integrating mode, 9.611 counts registered by the millivolt-time integrator were equivalent to 1 J cm^{-2} total energy. The integrator was used to measure irradiance for protracted periods, e.g. for the duration of an experimental incubation or for measurement of total daily irradiance.

c. Selenium photocell radiometer

This instrument was designed by Drew (1971) but has since been considerably modified, and so will be described here in some detail. It consisted basically of the detector, a selenium photovoltaic cell connected in series

with a microammeter. Two Cinemoid filters (Light Salmon, No. 9 and Steel Blue, no. 17, as used by Powell & Heath, 1964, combined transmission curve shown as curve a, Figure 2.8) mounted above the photocell altered its response from extremely green-sensetive (curve b, Figure 2.8) to an almost "equal energy" response between 400 and 650 nm, tailing off to 700 nm (curve c). The cell and filters were mounted below the collector which was constructed from ICI 040 Opal Perspex $\frac{3}{16}$ in. thick. The edges of this were shielded with black Perspex to ensure zero collection of radiation at angles of incidence of 90° and greater. When its response was measured as above for the Lintronic device, the collector showed a reasonable cosine response although exceeding the collection by a true cosine collector by 13% at an incident angle of 80° (Figure 2.7, curve c).

Since an irradiance of only about 2.5 mW cm^{-2} would produce full scale deflection of the galvanometer, several neutral density filters were constructed from Cinemoid No. 60 (Pale Grey) film, to attenuate high irradiances, thus maintaining the reading on scale. The instrument was calibrated using a standard light source from Instrument Specialities Corporation, Nebraska (ISCO) and Table 2.5 shows the conversion factors for μA to mW cm^{-2} for each filter combination.

Table 2.5 Selenium photocell radiometer - factors for conversion of galvanometer deflection with different filter layers

No. of layers of Cinemoid No. 60	Transmission %	μW cm^{-2} indicated by a reading of 1 μA		
		Dry	Wet	Submerged
0	100.0	5.0	4.2	5.2
1	30.0	16.7	13.9	17.4
2	9.0	55.6	46.3	57.9
3	2.7	185.0	154.0	192.0
4	0.8	625.0	521.0	651.0

The instrument was enclosed in a casing of ½ inch Perspex and was fully submersible. The instrument was calibrated dry, but when the filter units were wetted there was an increased response due to increased collecting efficiency. Thus, slightly lower conversion factors, shown in Table 2.5, were used when readings were taken above the surface with the filter units wet. When the instrument was used submerged, the "immersion effect" (Westlake 1965) was taken into account. This refers to the increase in back-scattering of light from within the opal collector when immersed deeper than one radius of the collector. With the collector at a depth of approximately 3 cm, this "subsurface" reading was close to 80% of the value obtained above the water's surface. Since the actual attenuation of PAR by this depth of water is negligible (and neglecting reflection at the water's surface), this implies an immersion effect of approximately 20% compared with the value of 22% found by Jerlov (1951) Westlake(1965) and Smith (1969). Factors for conversion of submerged readings to $mWcm^{-2}$ are shown in the last column of Table 2.5.

The instrument was used principally in measuring the attenuation of irradiance with depth in "irradiance transects". Readings were taken by a diver and the data recorded on a formica board. Transects were normally begun in shallow water and readings continued at approximately 5m intervals down the rock slope with the diver standing on the substratum, the meter being held at eye level, above the Laminaria hyperborea canopy. As the irradiance decreased with increased depth, the graded attentuation filters over the detector were changed. In water less than 3m deep, especially in sunny conditions, refraction of the sun's rays led to highly fluctuating galvanometer readings. Fluctuating readings were also obtained below a L.hyperborea canopy moved by the swell. In such cases, the mean position of the galvanometer pointer was taken. Transects were carried out only under relatively constant surface irradiance conditions. Underwater readings were expressed as a percentage of surface readings, which, due to the

application of the immersion effect correction, could be either subsurface or above-surface readings.

It should be noted that the relative insensetivity of this instrument between 650 and 700 nm will lead to a slight underestimate of PAR (400 - 700 nm) especially in surface measurements. This error will be negligible in submerged readings, however, due to the rapid attenuation of red light in the sea.

d. Selenium cell integrating radiometer

The instrument was designed by Drew (1972a) and was used in the present study with no further modifications. The detector was a selenium photovoltaic cell mounted beneath an opal Perspex (ICI 040 3/16 in) cosine collector. The cell was coupled to a coulometer consisting of a capillary tube containing a column of mercury broken by a small bubble of electrolyte solution. Wire electrodes were sealed into the ends of the capillary, in contact with the ends of the mercury column, and when a current passed, mercury was electroplated across the gap and the gap displaced by an amount proportional to the current passed. Gap movement was read against a millimetre scale fitted to the coulometer. In order to match the spectral characteristics of British coastal waters, the detector was fitted with a Cinemoid No. 24 (Dark Green) filter. Thus the instrument responded only to green irradiance with a peak at 510 nm and bandwidth of 88 nm (see Drew 1972a) which, at the surface, amounted to 17% of total PAR (400-700 nm) increasing to 18% at 3m, 22.5% at 12m and 26.5% at 18m in Jerlov's (1970) waters of coastal types 1-5. Table 2.6 shows the calculation involved in converting the gap movement in mm h^{-1} to total PAR in units of J $cm^{-2}h^{-1}$. The instrument was calibrated (against the selenium cell radiometer in preceding section) according to its subsurface response, so an "emersion" correction factor of 20% was applied to the dry surface readings; this included a correction of 7.5% for a small response in the red due to filter transmission

above 650 nm. Secondly, using the appropriate factor for each depth (Table 2.6, column 4), readings were corrected to represent total ambient PAR to give "corrected gap movements"(column 5) which were multiplied by a factor of 1.55 to yield irradiance between 400 and 700 nm expressed in J cm^{-2} h^{-1} (column 6).

The instrument was used extensively in <u>in situ</u> experiments at Puffin Island and Dunstaffnage.

Table 2.6 <u>Selenium cell integrating radiometer - factors for conversion of coulometer gap movement to integrated irradiance values</u>.

Depth	Gap Movement mm h^{-1}	Surface correction	Total correction	Corrected gap movement	Irradiance J $cm^{-2}h^{-1}$PAR
Above surface	1	0.8	100/17	4.71	7.30
Subsurface	1	1.0	100/17	5.88	9.11
3m	1	1.0	100/18	5.56	8.62
12m	1	1.0	100/22.5	4.44	6.88
18m	1	1.0	100/26.5	3.78	5.86

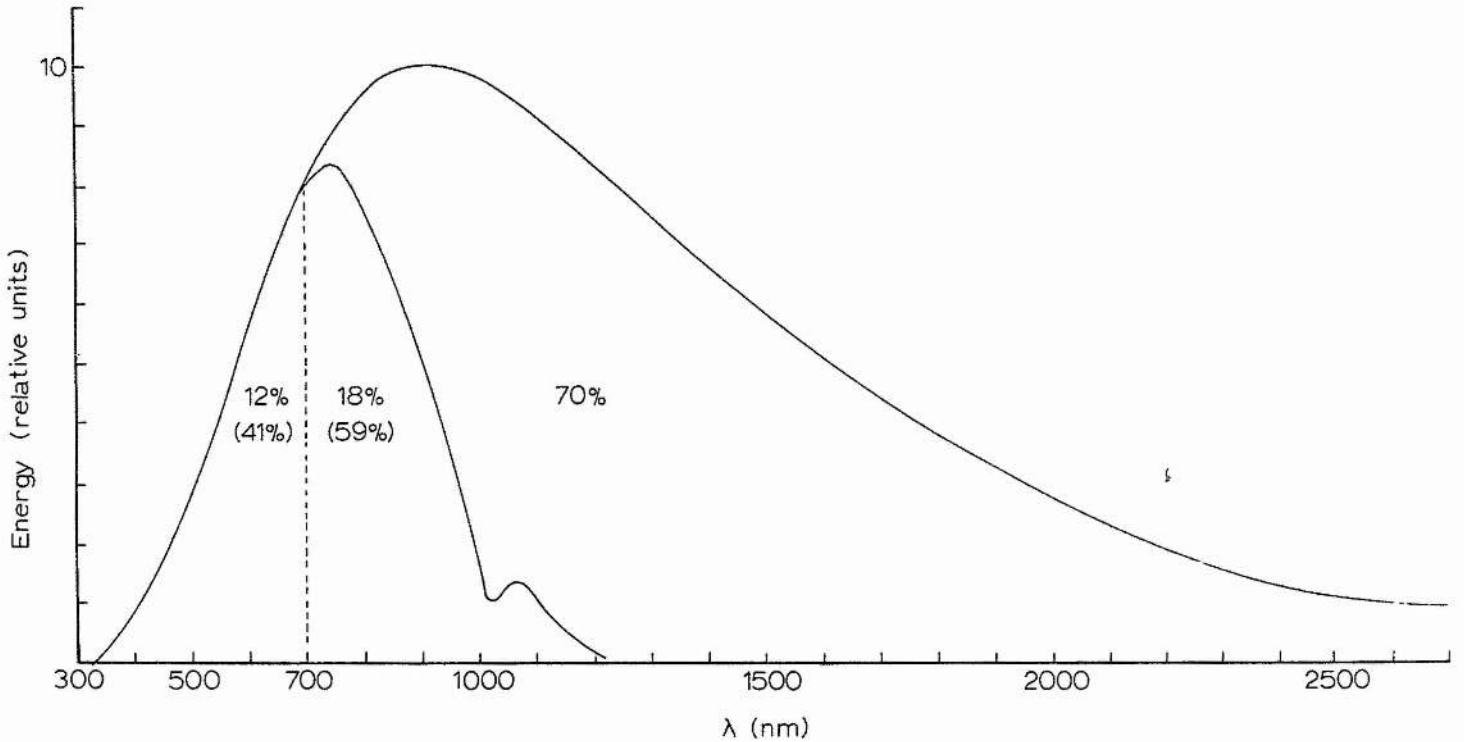


Figure 2.9. Spectral characteristics of tungsten-iodide light source; 70% of total energy is removed by the water filter, only 41% of the remainder is photosynthetically active.

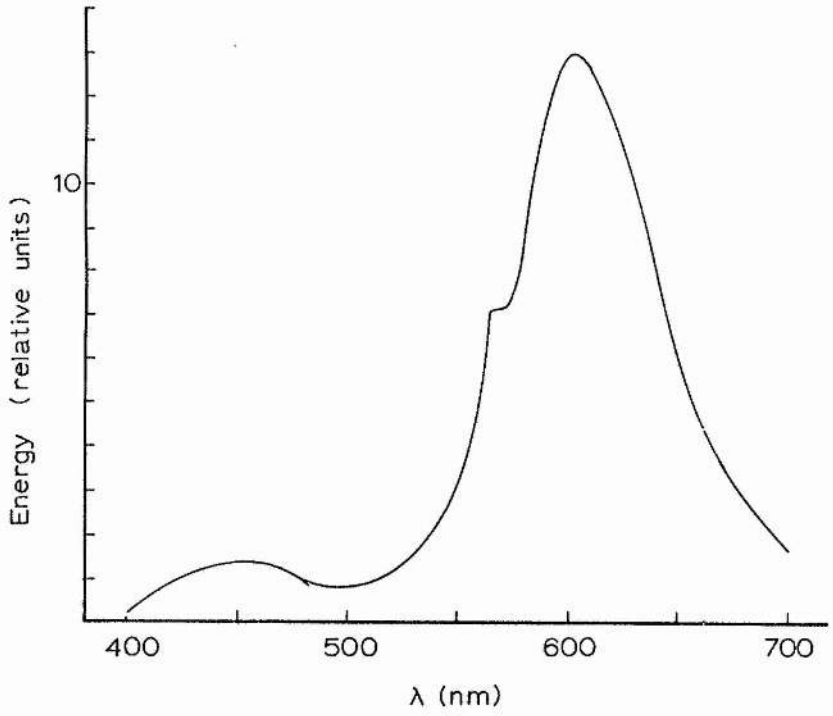


Figure 2.10. Spectral characteristics of fluorescent light source as measured with an ISCO spectroradiometer (redrawn from Jupp 1972).

7. Artificial light sources

a. Tungsten-iodide lamps

This light source was used in conjunction with the Gallenkamp constant temperature bath (Figure 2.5). Three Atlas K5 tungsten-iodide 240V, 1500W lighting tubes were arranged in a metal reflector so that one, two or three lamps could be used at once. The lamp housing was cooled by a small electric fan. A glass-bottomed tank containing 6 cm depth of flowing cold water was placed 20 cm below the lamps to reduce infrared radiation reaching the incubation bottles.

The relative spectral energy distribution of tungsten lamps of colour temperature approximately 3000°K (tungsten-halide lamps are closely similar) is shown in Figure 2.9 (from Phillips Electrical Limited leaflet PL 8123/2(372)). The combined effect of the 6 cm water filter and 0.3 cm of window glass (data for both from Wyszecki & Stiles, 1967, p.173) was effective in removing much of the infrared wavelengths accounting for 70% of the total irradiance. Of the radiation transmitted by the filter, only 41% was between 400 and 700 nm and can be taken to be photosynthetically active, thus only 12% of the total radiant output of the lamps was PAR, amounting to 14 m Wcm^{-2} at the level of the incubation bottles in the case of one lamp, 20mWcm^{-2} in the case of two and 28mWcm^{-2} when three lamps were used.

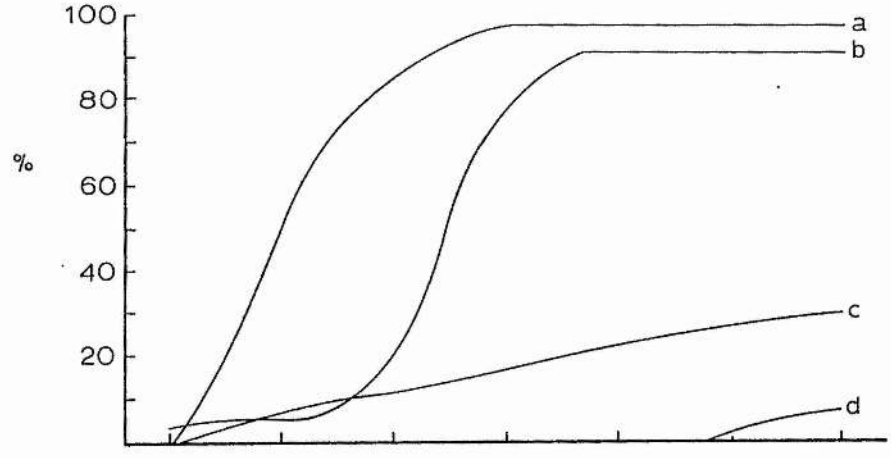
b. Fluorescent lamps

This source was used only in conjunction with the continuous flow incubation apparatus (see Figure 2.6), and consisted of an array of six fluorescent tubes, two "daylight", two "warm white" and two "Grolux" tubes. Each type of tube has a characteristic emittance spectrum and the combined relative spectral energy distribution curve is shown in Figure 2.10, as measured by an ISCO spectroradiometer. Almost all of the

the radiation was between 400 and 700 nm (i.e. PAR) and when used at a distance of 30 cm, this source produced an irradiance of 1.6 $mWcm^{-2}$ at the level of the plant specimens..

c. Optics of the incubation conditions

A complete characterisation of the optics of the field and laboratory incubations is not possible, but it is worthwhile to draw attention to certain general features which may affect the extent to which the experimental conditions approximate to the natural situation. The enclosure of all experimental samples in incubation bottles was probably the principal difference from the natural situation, but the cylindrical nature of these vessels was a reasonable approximation to the spherical assimilation chamber recommended as ideal by Sestak et al. (1971, p. 65). In the laboratory incubation chamber (p. 23) the filter windows (size dictated by the filter dimensions) were narrower than the chamber compartments which may have produced collimating or shading effects which were not accounted for. The tilting shaker had shallower compartments and wider windows, permitting a more even irradiance, although this type of shaking method was abandoned by Tseng & Sweeney (1946) who considered that the tilting action produced uneven irradiance. In the shaken experiments orientation was considered to be random and it was assumed that tissue was irradiated equally from all angles. In laboratory static, or field experiments, an attempt was made to orientate discs of relatively rigid species (e.g. Dilsea, Delesseria) in a horizontal position, but certain species (e.g. Porphyra, Ulva) were prone to curling, thus exposing variable areas for irradiation and introducing errors which could not be accounted for. When incubated in field platforms, samples were irradiated from a solid angle of close to 180°, but in the field incubation chamber the



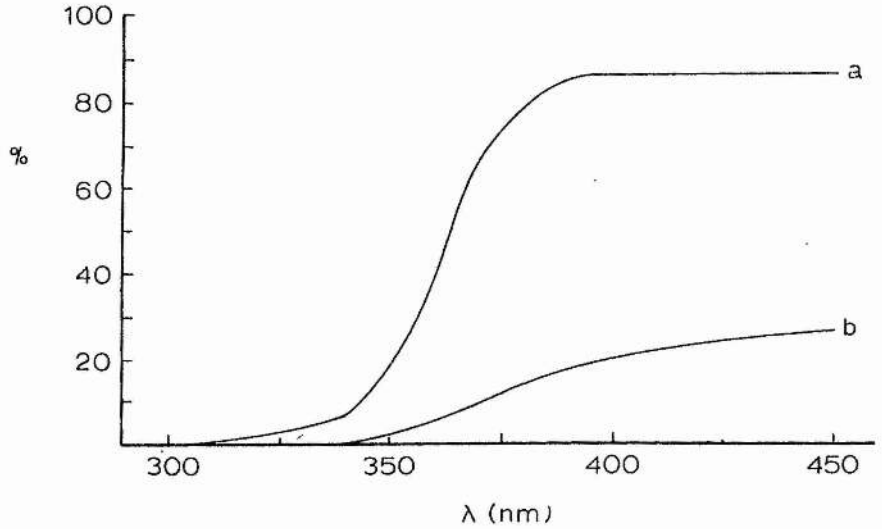


Figure 2.11. (upper). UV absorption spectra of "transparent" materials used in experimental incubations; a, incubation bottle glass; b, 3mm thick clear Perspex; c, Cinemoid No. 60 (Pale Grey) neutral density filter material; d, Cinemoid No. 23 (Light Green).

Figure 2.12. (lower). UV absorption spectra of combinations of "transparent" materials used; a, bottle glass + Perspex; b, bottle glass + Perspex + Cinemoid No. 60.

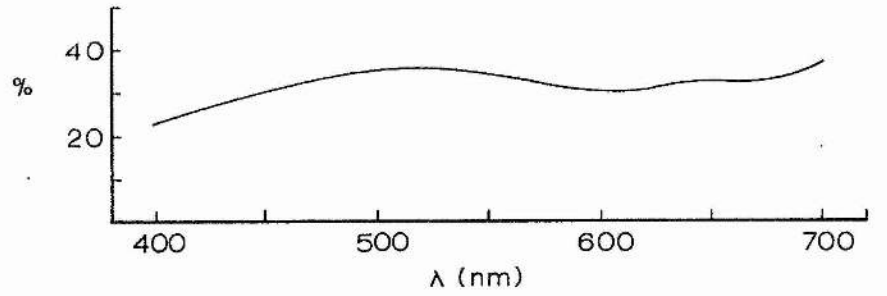


Figure 2.13. Absorption spectrum of Cinemoid No. 60 (Pale Grey) neutral density filter material.

angle was liable to be less than this due to shading by the walls and reflection from the Perspex window, at low angles of incidence. In the laboratory, irradiance was predominantly from above. The reflectivities of the backgrounds in the various incubation conditions were somewhat different, being low in the case of the gloss black laboratory incubation chamber, high in the gloss white field chamber and intermediate in the matt grey galvanised platforms. Thus the underside of tissue in each treatment would receive different levels of irradiance, as discussed by Sestak et.al.(1971, p.65) who recommended a matt black finish. In the present study however, it was felt that, used in the field chamber, matt black would have incurred overheating due to absorption of infrared radiation.

An important feature of the various "transparent" materials used in the construction of the incubation vessels and chambers was their absorption of short-wave radiation. Ultraviolet (UV) absorption spectra of the materials involved were measured with a Perkin Elmer 402 Ultraviolet-Visible spectrophotometer and are shown in Figure 2.11. The incubation bottle glass, with a 50% cut-off point at 325 nm, was considerably more transparent to UV than was Perspex with a 50% cut-off close to 360 nm. The Cinemoid No.60 "neutral density" filter had a more gradual cut-off consistent with its function, although generally transmitting less radiation here than in the PAR spectrum, shown in Figure 2.13 to be fairly "flat". Of all three materials, Perspex was proportionally most opaque to the middle UV (280-340 nm), the ratio of transmission at 325 nm to transmission at 400 nm being only 7% compare d with 52% in the case of the bottle glass and 27% in the Cinemoid No.60. A combination of glass and Perspex (see Figure 2.12) as used in the field incubation chamber, had a transmission similar to that of Perspex alone, while a combination of all three materials effectively removed all wavelengths progressively as the number of layers of Cinemoid No.60 was increased. Cinemoid No. 23 (Light Green) used in

chapter 7, p.211had no transmission below 420 nm (see Figure 2.11). The strong attenuation of wavelengths below 400 nm shown by these materials indicated that the incubation conditions were relatively "poor" in UV radiation compared with the natural siatuation where irradiance in this spectral region can be as high as 1 $mWcm^{-2}$ in surface sunlight in Britain (p.126).

Clearly, of prime importance are the very different spectral energy distributions of the artificial and natural light sources used, and these may be more significant than many of the above-mentioned factors.

8. Specific lamina area (SLA)

During the course of *in situ* experiments, specimens of algae were collected for determination of SLA. Where possible, samples of individual species were collected throughout their entire depth range, care being taken to select material which was both healthy in appearance and apparently representative for the different depths. Immediately after collection, whole individual plants (e.g. *Polyneura*, *Ulva*) or single fronds (e.g. *Delesseria*, depending on growth form, were selected and their outlines traced onto good quality paper having a uniform weight per unit area. Algal specimens were then transferred (quickly to avoid weight loss due to respiration) to individual numbered squares of aluminium foil and dried in a Calōr gas oven at 100°C. The dried tissues were carefully wrapped in their respective foil squares for transport back to St. Andrews laboratory, where they were again oven dried to constant weight at 100°C, and the dry weights determined. The numbered lamina shapes were cut out from the paper and the areas determined gravimetrically. (This method is described by Šesták et al. 1971, p. 519). Specific lamina area was the ratio, lamina area (cm^2) : lamina dry weight (mg).

9. Phycoerythrin extraction

Plants were collected by diving, carried back to the shore in black polythene bags where fragments (around 1g fresh weight) of the specimens were cut into 1 cm^2 pieces and transferred, in dim light, to 28 ml bottles containing distilled water, according to the method of Boney & Corner (1963). The specimens were then extracted at ambient temperature ($\sim 20^{\circ}C$) for one week and for a further four week period in the dark in a refrigerator at $4^{\circ}C$. At this point, the most concentrated extracts had the bright red colouring and orange fluorescence characteristic of phycoerythrin solutions, and the extracted tissue was green, indicating that extraction of the red pigment was relatively complete. This extraction procedure was not entirely satisfactory particularly in respect of length of time necessary for total extraction. However there are no universally accepted methods for extraction of phycoerythrin for quantitative assay, as there are in the case of the pigments soluble in organic solvents. In the present work, in an attempt to speed up the extraction process, grinding of algal material with quartz sand homogenised the tissue adequately but produced brown turbid extracts, a result also achieved by Haxo & Blinks (1950). Grinding of tissue in a ball mill for 24h met with the same result. Grinding tissue frozen by liquid nitrogen (see Jupp 1972) and ultra-sonic treatment, as used to fragment unicells (Brody & Emerson 1959) did not shorten the extraction time. A Wareing "Blendor" homogeniser fragmented tissue into pieces no smaller than could be achieved by grinding. More recent studies of the phycoerythrin content of red algae have utilised the Ten-Broek glass homogeniser (Moon & Dawes 1976) and the addition of polyvinylpyrrolidine (O'hEocha, pers. comm.) to increase yields of undenatured biliproteins.

The phycoerythrin extracts were decanted from the algal residue which was further washed with distilled water, the washings added to the extract

and made up to 25 ml standard volume. The algal residue was dried to constant weight in an oven at 100°C and the dry weight determined. Subsamples of the extracts were centrifuged in a small bench centrifuge for 15 min. The supernatant was decanted into quartz cells and the optical density measured on a Perkin Elmer 402 Ultraviolet-Visible spectrophotometer, between 190 and 850 nm. From a knowledge of $E^{1\%}_{1\ cm}$ = 81 at 569 nm, molecular weight of phycoerythrin of 290,000, (O'hEocha 1971) and from the observed optical density at 569 nm, the concentration of the solutions could be calculated per cent, then converted to µg phycoerythrin per ml solution, and finally to µg phycoerythrin per mg algal dry wt.

10. Ash, organic matter and energy content

Algal samples were collected and treated as above for specific lamina area, finally being dried to constant weight in the laboratory at St. Andrews, and ground to a fine powder with pestle and mortar. Approximately 1g subsamples were accurately weighed into nickel crucibles and ignited under oxygen at a pressure of 25 atmospheres in a Gallenkamp Ballistic Bomb Calorimeter CB370. Heat release was measured by the deflection of a galvanometer connected to a thermocouple in the machine. The galvanometer deflection was calibrated using thermochemical grade benzoic acid as a combustible substrate having an energy content of 26.45 J g^{-1} (6.32 cal g^{-1}). The residues remaining in the crucibles were considered to be the ash contents of the samples, and were weighed. The difference between ash weight and original dry weight of the sample was taken as organic matter content.

11. Bases, terminology and units used in expression of results

a. Photosynthesis and respiration rates

Šesták et al. (1971, p. 20) have reviewed bases and units for the expression of photosynthetic rates. It is generally recommended to use the base upon which the variation of the parameter under consideration is most dependent which in the case of photosynthesis, can be area or chlorophyll content. The former has been chosen for the present study as being easily measured and widely used in the literature. The unit of area chosen was the cm^2 since this was closest to the areas of thalli used in the experiments. Since experiments were usually of one hour's duration or more, the unit of time chosen was the hour (h). Although photosynthesis in terrestrial plants is usually expressed in terms of CO_2 uptake, the use of both HCO_3^- and CO_2 by marine plants makes C uptake more appropriate here for experiments using the ^{14}C method, the overall unit therefore being $\mu gC\ cm^{-2}h^{-1}$. Using the oxygen method, rates were expressed as units (μl) of O_2 evolved, the overall unit being $\mu lO_2\ cm^{-2}h^{-1}$. In cases where area was not measured or was difficult to measure, as in branched species (e.g. Laurencia) rates were expressed on a dry weight basis as $\mu gC\ mg^{-1}h^{-1}$ (^{14}C method) or $\mu lO_2\ mg^{-1}h^{-1}$ (oxygen method). In the ^{14}C method, if the dry weight was determined after alcohol extraction (as in the "indirect" method described above), the alcohol extracted dry weight was taken as the base. In the oxygen method too, some extraction of soluble substances was found to occur, and the ratio, unextracted dry weight : extracted dry weight could reach 1.47 in the oxygen method, and 1.96 in the ^{14}C method (shown by the SLA measurements before and after extraction, in Table 5.5). This means that rates expressed on an extracted dry weight basis could be up to two times the equivalent value expressed

on an unextracted dry weight basis.

Respiration rates were always originally calculated on a dry weight (after contact with Winkler reagents) basis, as $\mu l O_2\ mg^{-1} h^{-1}$, but for comparison with photosynthetic rates were frequently expressed also on an area basis. Since respiration does not depend on absorption of irradiance, it could be expected to be less dependent upon area than is photosynthesis (but see Chapter 8).

In order to compare results from both methods, rates expressed in $\mu l\ O_2$ were frequently converted to µgC, assuming that PQ (photosynthetic quotient, moles O_2 evolved : moles CO_2 fixed) and RQ (respiratory quotient, moles CO_2 produced : moles O_2 taken up) were equal to 1, using the factor $1\ \mu l O_2 \equiv 0.537$ µgC. Table 2.7 shows other interconversions based on PQ and RQ values of unity, used for conversion of photosynthetic and respiration rates from the present study and from the results of other authors. These factors are consistent with those of Sestak et al.(1971, p.27). The influence of PQ and RQ on rates of photosynthesis and respiration is discussed more fully below.

b. Irradiance

In accordance with recent recommendations (see Tyler 1973 a & b; Evans et al. 1975) the terminology and units employed in this thesis will be those of radiometry as opposed to photometry. As has been strongly emphasised frequently in the literature (e.g. Strickland 1958; Jerlov 1970; Šesták et al. 1971; Tyler 1973 a & b; Evans et al. 1975) photometry deals essentially with the "standard luminosity curve" which is the spectral response of the human eye i.e. energy between wavelengths of 400 and 700 nm with a pronounced peak at 555 nm, and is generally unsuited to considerations of radiation outside this spectral region, or monochromatic radiation within it. Radiometric units express absolute energy content regardless of wavelength. The hitherto much-used calories has now been largely discarded

Table 2.7 Conversion table for units for the expression of rates of photosynthesis and respiration*

$1\ \mu l\ O_2 \equiv 1.43\ \mu g\ O_2$

$\equiv 1\ \mu l\ CO_2 \equiv 1.97\ \mu g\ CO_2 \equiv 0.537\ \mu g\ C$

$1\ \mu g\ O_2 \equiv 0.699\ \mu l\ O_2$

$\equiv 0.699\ \mu l\ CO_2 \equiv 1.38\ \mu g\ CO_2 \equiv 0.376\ \mu g\ C$

$1\ \mu g\ CO_2 \equiv 0.508\ \mu l\ CO_2 \equiv 0.273\ \mu g\ C$

$\equiv 0.508\ \mu l\ O_2 \equiv 0.727\ \mu g\ O_2$

$1\ \mu g\ C \equiv 3.67\ \mu g\ CO_2 \equiv 1.86\ \mu l\ CO_2$

$\equiv 1.86\ \mu l\ O_2 \equiv 2.66\ \mu g\ O_2$

* These data are based on the assumption that one mole of any gas occupies 22.4l of volume at NTP ($0^{\circ}C$). When considered at $15^{\circ}C$, the factors in this table would have approximately 5% error.

in favour of metric units, thus, in the present work, irradiance will generally be expressed as mW cm^{-2}, followed if applicable by the letters PAR to denote photosynthetically active radiation, i.e. wavelengths from 400 to 700 nm. When integrated over a period of time, irradiance has been expressed as J $cm^{-2}h^{-1}$ and, since a joule per second is equivalent to 1 watt, for periods of uniform irradiance, 1 J $cm^{-2}h^{-1}$ = 0.278 mW cm^{-2} and, conversely, 1 mW cm^{-2} = 3.6 J $cm^{-2}h^{-1}$.

Certain terms should not, strictly, be used when referring to irradiance, viz.,

Photometer - an instrument for comparing the luminous intensities of two sources of light.

Illumination (= illuminance) - photometric term denoting irradiation by "visible" radiation.

Light - "visible" radiation

Intensity (= radiant intensity) - refers to energy emitted by a source, not to energy received by a surface (Westlake 1965; Collocott 1971).

These terms are still in frequent use in the literature, often wrongly, in conjunction with radiometric terms (e.g. Stanton 1973: Steemann - Nielsen 1973). The unit of illumination is the "lumen" which is a "specially defined watt" (Tyler 1973 a) equal to one candle power per second. There are 680 lumens per watt when the radiant source has the standard luminosity curve. For other radiant sources, however, the value of the lumen is different and a range of values used in this thesis to interpret data in the literature is shown in Table 2.8 including information on "lux" , (lumens m^{-2}) and the older "foot-candle", still appearing in published work (e.g. Mathieson & Norall 1975).

Table 2.8. Approximate energy equivalents of one kilolux* (From Westlake 1965).

Light Source	mW cm^{-2} Total	Visible
Standard candle	0.140	0.140
White fluorescent lamp	0.348	0.279
Daylight	0.906	0.419
Tungsten filament lamps	4.186 - 5.581	0.419 - 0.558

* 1 lux = 1 lumen per m^2

1 lumen = 1 candle power per second

1 candle power is a unit of luminous intensity equivalent to the Sl unit, the candela and approximately equivalent to 0.0015 Joules.

1 foot candle = 1 candle power per square foot per second

= 1 lumen per square foot

= 10.76 lux.

1g calorie at 15°C= 4.1855 Joules

1 langley per minute = 1 g cal cm^{-2} min^{-1} = 69.8 mW cm^{-2}

Although the term "light" as has been stated is strictly speaking a photometric term, it will be used in this thesis in an uncritical "common usage" sense to denote radiation of the approximate spectral region 400-700 nm, i.e. visible or photosynthetically active radiation.

Details of the relationships between photons or quanta, and radiant energy appear on pp.127 and 226.

c. Depth

The expression of depth is very important in aquatic environments and is central to some of the topics discussed in this thesis. The fluctuation of water level due to the tides adds a complication not encountered in fresh-water ecosystems. Workers in the littoral zone have generally expressed position on the shore profile with respect to the various states of tide, extreme high water springs (EHWS), extreme low water springs (ELWS) etc. In sublittoral work, it is important to convey the height of the water column above the point under consideration. Several workers have expressed depth below ELWS (e.g. Kain 1960; Smith 1967; Jupp & Drew 1974) or other extreme low water measures such as "lowest lower low water", LLLW (Druehl 1967), chart datum, CD (Svendsen & Kain 1971) or lowest astronomical tide, LAT (Kain 1971, 1976). A disadvantage of these observations is that the depth below ELWS is a measure of the shallowest extreme that sublittoral plants are exposed to, and gives no indication of the mean height of the water column above the plants. This was emphasised by Kain (1971) who suggested stating depths below LAT as well as below mid-tide level, so that regions with different tidal ranges could be compared. In the present study, depths have been expressed as metres (m) below mid-tide level, and the tidal range of the location is given (see Chapter 5). At Eilean Hoan, the tidal range at spring tides was actually measured, but at all other sites, the range was

obtained from the Admiralty Tide Tables (Admiralty 1974). In work concerning the Mediterranean Sea, where tidal range is usually much less than 1m, the tidal range has frequently been ignored and depths can be assumed to be measured with respect to mid-tide levels (e.g. Larkum et al 1967; Giaccone 1972). Even in work dealing with oceanic shores, however, reference to tidal range has freqently been omitted (e.g. Michanek 1967; Mann 1972; Mathieson & Norall 1975). Certain workers have used mid-tide level but have not specified tidal range (e.g. Aleem 1973).

e. SI units

In this thesis, units were used which were closest to the order of magnitude of the parameters being measured, and in doing so, many are not recommended SI units - thus, photosynthetic rates have been expressed as $\mu g\ C\ cm^{-2}h^{-1}$, rather than $kg\ C\ m^{-2}s^{-1}$. All units used are, however, metric and are compatible with and easily converted to, SI units.

12. Statistics

Simple statistical analyses of the data, including standard deviation standard error, t-statistic, correlation coefficient, linear and curvilinear regression were performed according to standard texts (e.g. Campbell) and utilising the Hewlett-Packard "Stat-Pac" programmes in conjunction with a HP65 card-programmable calculator.

In all *in situ* experiments and most laboratory ones, pairs of replicate samples were used, for each species in each treatment, and the pair mean calculated for each species. Graphs and histograms representing only one experiment show the pair mean ± standard error of the mean (i.e. ½ the range). When more than one experiment is represented, values shown are the mean of the pair means from each experiment, ± standard error of the mean.

In tables, means ± standard errors are also shown, n denoting the number of experiments represented. In tables containing values derived by the application of conversion factors to original data (e.g. conversion of µl O_2 to µg C, or of photosynthesis on dry weight basis to area basis) the converted values do not carry the standard error.

13. A critique of the ^{14}C and oxygen methods used in the measurement of photosynthesis

In the course of the present work it was apparent that values obtained using the ^{14}C method were consistently higher, by a factor of around two, than values obtained when the oxygen method was used. Clearly, the two methods measure different parts of the photosynthetic process, and if incorrect assumptions are made about the interrelations of these two parts, then the rates calculated from each method will necessarily be in disagreement. One such assumption, that the photosynthetic quotient = 1 (where PQ = ΔO_2 :- ΔCO_2, see p. 53) is indeed open to question and will be discussed below. However, there are certain methodological considerations as well as physiological ones which should also be discussed when considering the comparibility of these two methods. Using techniques similar to those of the present study, Forbes (1975) found that the ^{14}C method consistently gave photosynthetic rates in the red algae Porphyra umbilicalis and Rhodymenia palmata, which were close to twice the values obtained with the oxygen method under the same conditions, using a PQ of unity. Drew (pers. comm.) however has found that the discrepancy between the results obtained with these two methods is variable according to the physiological state of the algal experimental material. This may be due to changes of the two processes measured, relative to each other. Such change was found by Ryther & Vaccaro (1954) to occur in phytoplankton cultures, as the age of the

cultures increased. Several works exist comparing the results of primary productivity measurements obtained using both methods in the study of phytoplankton (e.g. Steemann-Nielsen 1952; Ryther & Vaccaro 1954). However, the use of both techniques in the study of macroalgae is less well documented and there follows a discussion of possible methodological and physiological explanations of the disparity between the methods.

a. Possible sources of error in ^{14}C method

Specific radioactivity of seawater samples

This is a measure of the "average radioactivity" of the organic carbon available to the alga, and is expressed in units of cpm μgC^{-1}. If the specific activity was underestimated, an overestimate of photosynthetic rate would result, and vice versa.

Errors in the assessment of specific radioactivity could occur if the inorganic carbon content of the seawater was incorrectly estimated. In the present work, carbon content was taken to be 30μgC ml^{-1} in the Mediterranean and 26μgC ml^{-1} in Britain, these being the theoretical concentrations for waters of the respective salinities. Estimates of the carbon contents of seawater in the Mediterranean by the present author, and in British waters by Drew (unpublished) conformed closely to the theoretical values, indicating that this was not a major source of error.

Since specific activity could only be overestimated if some form of contamination was consistently occurring, this seems an unlikely source of error. Underestimation, however, could possibly occur either by (a) adsorption of ^{14}C onto the walls of incubation vessels (Pomeroy, 1961, advocated the use of large volume containers to reduce the surface : volume ratio) (b) some form of breakdown of ^{14}C bicarbonate by microorganisms, resulting in release of $^{14}CO_2$, or (c) by release of $^{14}CO_2$ by inorganic means, i.e. equilibration with $^{12}CO_2$ in the air. This last occurrence

has been foreseen, and avoided, by Craigie (1963) who added NaOH to water samples at termination of incubation to ensure a high pH and discourage evolution of $^{14}CO_2$. Thus, any error in estimation of specific radioactivity is liable to be an underestimate, resulting in overestimates of photosynthetic rate.

Self-absorption corrections made to counts of water precipitates and whole and macerated tissue samples

Jitts (1963) pointed out that theoretical approximations of self-absorption curves to hyperbolic or exponential functions (as in the present study) can lead to errors at densities of <1 mg cm^{-2}. Since the correction itself is fairly small at such densities (Table 2.2) this was probably not a serious source of error. However, with increase in density, there is a decrease in accuracy of the absorption correction. In the present method water precipitates and whole tissues were usually in the range 1-3 mg cm^{-2}, and it is doubtful if the corrections employed introduced a high error.

The present method (Table 2.2) assumed that less radiation was self-absorbed than was assumed by Steemann-Nielsen & Aabye-Jensen (1957) in the original method (e.g. , they assumed 32% of total counts were collected at a density of 10 mg cm^{-2}, compared with 40% as shown in Table 2.2). This would result in an overestimate of photosynthetic rate. Conversely, an insufficient self-absorption correction applied to tissue samples would result in an underestimate of photosynthetic rate.

Contamination of plant samples by inorganic ^{14}C

In the present method, algal tissue was briefly washed in distilled water before extraction in alcohol, or drying on a planchet. Although this would remove much excess inorganic ^{14}C, the alcohol extracts and dried tissues were further treated, prior to counting, with glacial acetic acid

to drive off $^{14}CO_2$. If this procedure was inadequate in removing all inorganic ^{14}C, the tissue and alcohol extracts would give excessively high count rates and consequently indicate high phytoplankton rates. Removal of inorganic ^{14}C from filters in the phytoplankton techniques have involved fuming with HCL gas (Steemann-Nielsen & Aabye-Jensen 1957) and washing with dilute (0.001 M) HCL (Ryther & Vaccaro 1954). However, control "de-activation" of radioactive water samples using the glacial acetic acid method resulted in background readings, indicating that this method was fully adequate.

Dark fixation of carbon

As do terrestrial plants, and phytoplankton, marine macroalgae fix carbon into organic compounds in the dark, and it is a premise of the ^{14}C method that, being independent of photosynthesis this process continues, unchanged, in the light (Steemann-Nielsen & Aabye-Jensen 1957). Initial experiments with phytoplankton indicated that dark fixation was of the order of 1-2% of saturation photosynthetic rate (Steemann-Nielsen & Aabye-Jensen 1957; Ryther 1954). In the present study however, dark fixation rates of up to 10% of saturation photosynthetic rates were found. Morris et al. (1971 a,b) found that in phytoplankton cultures, dark fixation decreased from 71% to 0.3% as the cell suspension density increased. Morris et al. (1971 a) however recommended that there was no justification for assuming that dark fixation proceeded at the same rate in the light, and, for phytoplankton studies at least, recommended that dark fixation rates were not subtracted from photosynthetic rates. Since in the present method, dark control incubations were always carried out, and the rates subtracted from the relevant photosynthetic rates, this procedure would lead to an underestimate of photosynthesis, according to Morris et al. (1971 a).

6. Possible sources of error in the oxygen method

Quantitative estimation of dissolved oxygen content

This is the basis of the oxygen technique, and if the conversion from titre of sodium thiosulphate to microlitres of oxygen were in error, there would be a corresponding error in estimates of photosynthetic (and respiration) rates. Drew & Robertson (1974a, & Appendix 1) found, however, that the method gave estimates of the oxygen content of seawater which were in accordance with theoretical values (e.g. Richards & Corwin 1956).

Reactions of algal tissue with oxygen

By placing algal tissue samples in incubation bottles with seawater and processing with the Winkler technique immediately, it was shown that no non-metabolic uptake of oxygen occurred (e.g. by oxidation of exuded mucilages, etc) at least in the short-term.

Photosynthesis and/or respiration by microorganisms

Photosynthetic rates of up to 26 μg C m^{-3} h^{-1} have been recorded for phytoplankton in seawater in the temperate zone (Wallen & Geen 1971). Such rates could account for up to ∿10% of the oxygen produced in an incubation bottle containing a macroalgal sample of low photosynthetic capacity, leading to a slight overestimate of photosynthesis for the tissue. However, on several occasions, when seawater controls were assessed for oxygen content at the commencement of an experiment, and also after incubation in the light and dark, changes in oxygen contents were negligible in comparison with the changes incurred by macroalgal samples. Sargent & Lantrip (1952), however, estimated that oxygen uptake in dark bottles containing discs of Macrocystis pyrifera was increased by bacterial oxidation of exuded mucilage. They assumed that one half of the total oxygen uptake of such bottles was due to this cause.

Effects of surfaces

Laevastu et al.(1965) found that, in phytoplankton productivity experiments, oxygen uptake (in the dark) increased in direct proportion to the surface area of inert material (glass rods) present. In the present work, this could lead to higher respiration and lowered photosynthesis estimates in algae posessing high surface:volume ratios.

As Drew and Robertson (1974 a & Appendix I) found, the use of plastic bottles in this technnique is inadvisable due to inward diffusion of oxygen.

c. Possible physiological sources of error in both methods

Photosynthetic quotient (PQ $\doteq \Delta O_2 : -\Delta CO_2$)

After analysing several phytoplankton species for carbon, oxygen and nitrogen content, Ryther (1956b) concluded that the PQ for long term growth must be greater than unity, and he suggested a value of 1.25. Similarly, Westlake (1963) recommended the use of PQ = 1.2 for plants growing in unfavourable conditions and 1.25 when conditions were optimal. In the present study it is probable that, considering short-term photosynthesis of the red algae studied, most photosynthate is in the form of carbohydrate (see Majak et al. 1966) and that a PQ of unity is thus a reasonable assumption. If the PQ was in reality around 1.25, and a PQ of 1 was used in conversion calculations (as here - Table 2.7) the carbon fixation rates calculated from oxygen method experiments would exceed the true values. This error does not occur in the direction expected from the results in the present work, which implied that carbon fixation rates estimated from oxygen method experiments were, if anything, underestimates of the true values, since they were less than those of the ^{14}C method.

Respiration in the light

Dark or mitochondrial respiration, by definition occurring in the dark, was for long presumed to occur also in the light at a similar rate. This assumption, on which the basic light-dark bottle technique was based (Gaarder & Gran 1927) led to computation of gross photosynthesis by adding dark oxygen uptake rate to rate of oxygen evolution in the light. This method is now wholly invalidated (Sestak et al.1971, p.20) by the revelation that dark respiration does not invariably proceed unchanged in the light, and that a separate form of respiration, photorespiration, occurs in the light exclusively. Mitochondrial respiration is considered to result from the complete oxidation of an organic compound (generally carbohydrate) to CO_2 and H_2O, with molecular O_2 as the ultimate electron acceptor (Gibbs 1962). In that the respiratory substrate consists of stored material, oxidised by the Embden Myerhof Parnas and other pathways, this form of respiration is not immediately dependent upon photosynthesis.

Recently, however, certain effects of quantity and quality of light upon dark respiration have been demonstrated. Hoch et al.(1963) found that oxygen uptake by Anacystis nidulans (Cyanophyta) was less at low irradiances than in the dark, the phenomenon being termed the Kok effect (see Heath 1969), since discovered in Chlamydomonas (Chlorophyta) by Healey & Myers (1971) at irradiances less than 0.3 m W cm^{-2}. Kowallik (1967) found that low-level irradiance of blue wavelengths resulted in reduced O_2 uptake in Chlorella (Chlorophyta). These are both factors which could be of importance to algae growing in the low-level blue-green irradiance at the lower extreme of the photic zone. At higher irradiance levels, rate of mitochondrial respiration is generally held to be the same as (Raven 1972) or less than (Brown & Tregunna 1967) in the dark. However, Mangat et al.(1974) found mitochondrial respiration in the light to be four times the rate in the dark,in Phaseolus leaves.

Because it involves stored carbohydrates, dark respiration is held to result in no great loss of ^{14}C fixed during short-term experiments ($\sim$4h duration). However, Steeman-Nielsen & Aabye-Jensen (1957) suggested that dark respiration was so low compared with photosynthesis (in photoplankton) that loss of ^{14}C would be negligible in any case. Steemann-Nielsen (1955) found that phytoplankton lost 0.6-3% of previously fixed ^{14}C, through respiration in the dark, and concluded from this that the ^{14}C method measured something between gross and net photosynthesis. Jupp (1972), after incubating Laminaria hyperborea in ^{14}C for two hours in the light, found that 0.2% was lost after a third hour of incubation in the dark rising exponentially to 6.6% after 4h in the dark. In the light 75% of this lost ^{14}C was apparently re-fixed, leaving $\sim$2% total loss after 4h. Thus it appears that in an experiment on only 1h duration, this method measures effectively gross carbon fixation. At an incubation time of 4h, something between net and gross fixation will be measured, tending towards net measurement at incubation times in excess of this, as ^{14}C becomes increasingly incorporated into the pool of dark respiratory substrate.

Clearly, since the oxygen method integrates the evolution of oxygen by photosynthesis and the uptake of oxygen by respiratory processes, there can be no dispute that this method measures net oxygen evolution, and hence, net photosynthesis.

Considering, briefly, photorespiration - defined by Tolbert (1974) as "light dependent oxygen uptake and carbon dioxide release occurring in photosynthetic tissues". Photorespiration involves the oxidation of recently-formed photosynthate, generally glycolate, and so would appear to concern the ^{14}C method more than does mitochondrial respiration in short-term experiments. However, because it is linked directly to photosynthesis it cannot theoretically outpace photosynthesis, and a light compensation point does not occur with photorespiration. Because it proceeds

concurrently with photosynthesis, and does not involve stored substrates, photorespiration incurs no net loss of carbon from the plant but is merely a wasteful part of the photosynthetic process. Thus, in the ^{14}C method, it is probable that ^{14}C has quite a high turnover rate by being fixed by photosynthesis, "lost" as $^{14}CO_2$ by photorespiration, and re-fixed by photosynthesis. Assuming that oxygen production by photosynthesis and uptake by photorespiration are both directly related to irradiance, photorespiration should not affect measurement of photosynthesis by the oxygen method, which will still measure a net rate of oxygen evolution.

Thus, both ^{14}C and oxygen methods will be equally unaffected by photorespiration. Photorespiration has been found to occur in macroalgae by Brown & Tregunna (1967) and discussed with regard to the algae in general, by Jackson & Volk (1970), Tolbert (1974) and Chollet & Ogren (1975).

Excretion of photosynthate

Excretion of recently-formed photosynthate would lead to underestimation of photosynthesis, measured by the ^{14}C method. Sieburth (1969) found that exudation of organic carbon from macroalgae could reach maxima of 5.4 μg $mg^{-1}h^{-1}$ in Ascophyllum nodosum (Phaeophyta) and 2.0 μg mg^{-1} h^{-1} in Ulva lactuca (Chlorophyta) but was much lower in the rhodophytes Chondrus crispus (0.4 μg mg^{-1} h^{-1}) and Polysiphonia lanosa (0.04 μg mg^{-1} h^{-1}). However, Khailov (1969) found no correlation between exudation rate and taxonomic position, Rhodymenia palmata (Rhodophyta) exuding 9.8 μg mg^{-1} h^{-1} whilst Laminaria saccharina (Phaeophyta) exuded only 1.7 μg mg^{-1} h^{-1}. The value for Rhodymenia given would account for 47% of the photosynthesis measured in this species in the present work, implying that the measured rate should in fact be 1.5 times the value found. Since Rhodymenia had a very high carbon fixation rate as determined in the present study however, it is doubtful whether such a correction would be fully justified.

and Burlakova

Drew (pers. comm.) studying the brown alga Laminaria hyperborea with the ^{14}C technique as used in the present study, found no radioactivity in organic constituents in the bathing water from experimental incubations of 6h duration. In a study of the direct effects of exudation on the measurement of photosynthesis in 24 species of phytoplankton, using the ^{14}C method, Nalewajko (1966) found that only ~5% of fixed ^{14}C was excreted as organic carbon during a 1h duration period. Ryther & Menzel (1965) found that in phytoplankton, photosynthesis measured by the ^{14}C method was the same as that computed gravimetrically from growth measurements, concluding that if excretion did occur, it must be of immediate products of photosynthesis.

Thus, although the findings of Sieburth (1969) and Khailov (1969) do indicate that substantial exudation or excretion of organic carbon can occur in macroalgae, it appears that this may not incur a significant loss of ^{14}C-labelled compounds in short-term experiments. If it were to, it would mean that rates measured by the present technique were underestimates. The oxygen method should be unaffected by exudation.

Photooxidation processes

Franck & French (1941) found that oxygen uptake by Hydrangea was higher in the light than the dark, and, since this occurred in boiled as well as live tissue, they concluded that it was due to the photooxidation of intermediate products of photosynthesis, sensetised by chlorophyll. Griffiths et al. (1955) found that carotenoid-less mutants of photosynthetic bacteria underwent photooxidation of their bacteriochlorophyll when irradiated under aerobic conditions, and proposed a protective function for caroteniods, by this means, in all plants. Cholnoky et al. (1956) described how caroteniods in higher plants underwent reversible oxidation as part of the oxygen transport system of green plant organs. It is

possible, then, that oxygen uptake by such processes may occur in the algae studied (without production of CO_2 and loss of ^{14}C) and would reduce the estimated rate of photosynthesis by the oxygen method, without effect upon the ^{14}C method. Such effects would be most pronounced at high irradiances. McAllister (1961) found that in the coccolithophore Syracosphaera, photosynthesis measured by the oxygen method was similar to that measured by the ^{14}C method at low irradiances but dropped dramatically at irradiances above ~10 mW cm^{-2} PAR; using the ^{14}C technique photosynthesis remained at a steady rate above 10 mW cm^{-2}. He suggested that this was due to uptake of oxygen by photooxidation processes occurring at the higher irradiances. Drew (pers. comm.) However, found that photosynthetic rates of L.hyperborea measured by the ^{14}C method were consistently higher, by a factor of ~2, than rates determined using the oxygen method, at both low and high irradiances, indicating that photooxidation was not the cause for the discrepancy between the methods.

Thus, of all the above-cited possible methodological and physiological causes for the observed discrepancy between ^{14}C and oxygen estimates of photosynthetic rate, none emerges as a single explanation. In the present work the ^{14}C method has been taken to measure near to gross photosynthesis, and the oxygen method to measure net photosynthesis. These have been interconverted using a PQ of unity, although, for long term growth, the PQ value is probably closer to 1.2.

Due to the uncertainties concerning respiration in the light, in no case have rates derived from the ^{14}C method been "converted" to net rates by subtraction of dark respiration rates, nor have rates derived from the oxygen method been converted to gross fixation by addition of a dark respiration rate.

Qualitatively, the two methods were in very close agreement, as will be shown, and the quantitative disparity must be taken as a reminder of the difficulties associated with indirect measurements of physiological processes. It may be that the variability of the discrepancy between the two techniques could be of use in investigating its nature.

CHAPTER 3

Factors affecting the supply of oxygen and inorganic carbon

CONTENTS

1. Introduction

The uptake of solutes at submerged plant surfaces is limited primarily, in some cases totally, by the concentration of these solutes at the absorbing surface. This can be modified in two ways, firstly by alterations in the concentration in the bulk of the solution and secondly, by alterations in the layer of solution adjacent to the absorbing surface. An example of limitation of the former type is seen in nature in the decline of growth of phytoplankton blooms due to the depletion of certain nutrients in the sea. Such depletions occur when the ratio of mass of living organic matter to volume of bathing medium is high. This is a feature of great concern in experimental work involving the enclosure of plant material in vessels of finite volume. The second type of limitation is universally applicable to uptake situations. A region of uptake, if the rate of uptake is sufficiently high, quickly forms a layer of medium around it which is depleted of the molecular species concerned, and this rather ill-defined region is termed a "boundary layer". The relative thickness of such layers and therefore the steepness of the concentration gradient across them depends inversely on the rate of replacement of the solute which is being taken up. This in turn depends on the degree of mobility of the solute molecule or ion and, importantly, on the degree of movement of the medium. Thus, well stirred or mixed conditions effectively raise the concentration of the solute concerned near the site of uptake. As early as

1926, Ruttner had said that moving water was "not absolutely, but rather physiologically, richer in oxygen and nutrients" (quoted from Whitford 1960). The movement of solutes across boundary layers (or unstirred layers) is solely by molecular diffusion which, although vital at this "micro" level, is of little significance as a mode of mass transfer in large bodies of water because it is effectively very slow when operating over distances greater than about one centimetre (Hutchison 1957; Riedl 1971). The present chapter describes work designed to elucidate the extent of limitation of oxygen and carbon supply produced by the experimental methods, particularly with regard to the unstirred in situ experiments, and attempts to relate the findings to the supply situation in nature. The effects of boundary layers under static conditions have led to a general agreement among physiologists that experiments should be conducted under agitated conditions. Doubts are frequently expressed, however, as to whether the shaking conditions, frequently arbitrarily imposed, truly reflect the natural situation or result in either over or under- estimates of metabolic rates (Doty & Oguri 1958; Gessner & Pannier 1958; Kain et al. 1975).

The literature presents a few studies which correlate the supply of oxygen and inorganic carbon with physical factors such as water movement in marine environments (Jones 1959; Conover 1967; Smith & Marsh 1973; March 1974), and many dealing with fresh water environments (e.g. Odum 1956a; Whitford 1960, 1964; Whitford & Schumacher 1961; Blum 1962; Schumacher & Whitford 1965).

Clearly the growth of submerged plants will be greatly influenced by the effects of water movement on availability of nutrients, but growth is also affected by the grosser physical aspects of water movement which may be a combination of physiological and mechanical factors. Studies of water movement in the sea and its direct relevance to the growth and form of macroalgae have been undertaken by Carstens (1968), Jones & Demetropolous (1968), Muus (1968), Charters et al.(1969), Doty (1971). The subject has

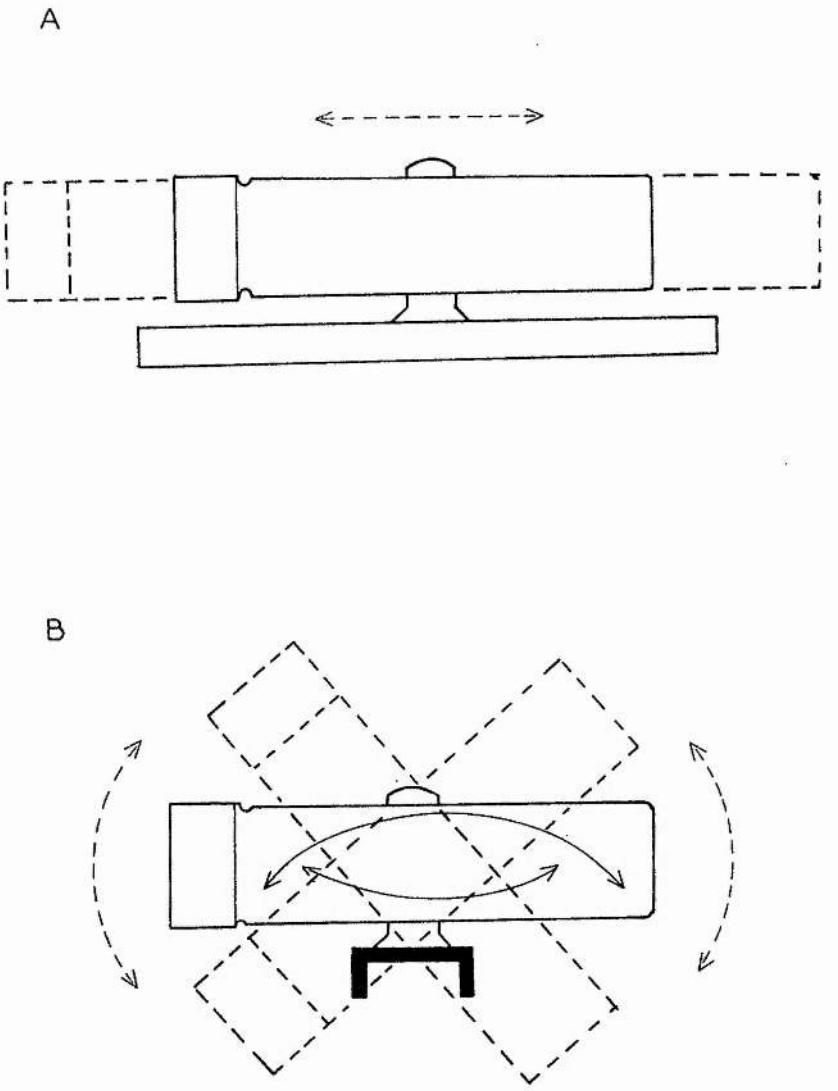


Figure 3.1. Schematic diagram of two shaking modes; A, horizontal; B, tilting. Bottle movement shown by broken arrows, water movement by solid arrows.

been reviewed by Schwenke (1971). These workers were concerned specifically with the measurement of water movements on a small scale much neglected by oceanographers whose interests lie in the behaviour of large-scale ocean currents. The measurement of water movements affecting plant growth present many problems, especially of instrumentation. Thus the micro-climate of the marine plant surface is a somewhat unknown territory compared with the rather well documented analogy of the terrestrial aerial leaf surface (Evans 1963; Heath 1969; Monteith 1973). Studies of the microenvironment of marine invertebrates have been made by Ott (1967), Riedl (1969,1971) and Riedl & Forstner (1968), yielding valuable information about boundary layers, which may be of use to the plant physiologist also.

Associated with the physiology of uptake of "material" resources is the "morphological" factor. Arber (1920) early correlated aquatic leaf form with absorptive function and more recent marine studies have attempted to do the same (Odum et al. 1958; Norton 1969).

2. Note on methods

Previous work in this laboratory (Jupp 1972) involved the incubation of algal material in 28 ml incubation bottles using the ^{14}C method to measure photosynthetic rate. The Gallenkamp constant temperature bath was used (see Chapter 2) with the incubation chamber mounted in the carriage of the shaker mechanism as described. The shaking movement of the bottles is shown in Figure 3.1A. Isotope experiments can be conducted thus, with an air bubble (approximately 3 ml volume) present, causing effective mixing of the seawater medium as the carriage oscillates. The use of the Winkler oxygen technique necessitated using bottles with gas bubbles totally excluded

and consequently there was doubt as to the effectiveness of the shaking method. A simple test involved the injection of a small quantity (about 0.1 ml) of concentrated potassium permanganate solution (deep purple colour) into a bottle fitted in the shaker. At normal shaking frequency of 1 to 1.2 cycles s^{-1} the distribution of the coloured tracer in the bottom of a completely filled bottle remained unchanged for several minutes whilst the contents of a bottle with air bubble present were fully mixed almost instantly. Thus the horizontal action was not initiating any relative movement between the glass vessel sides and the contained water. Only loosely fitting, relatively dense algal discs were observed to move, due to their inertia, in such shaking conditions. The shaker was redesigned to allow the bottles to be shaken in a tilting manner. The dye technique showed that the inertia of the contained water relative to the movement of the bottle walls (see Figure 3.1B) initiated complete mixing almost instantly, and presumably continually thereafter.

3. Photosynthesis and respiration rates measured under static and agitated conditions

a. Photosynthesis using horizontal shaker (^{14}C method)

An experiment was conducted to show whether the physical stasis indicated by the coloured tracer experiment was in fact limiting the measured photosynthetic rate in "shaken" bottles. The horizontal shaker unit was modified so that six replicate bottles could be shaken and six remain unshaken under otherwise similar conditions. It was assumed that water movement in the static bottles was not induced by vibration of the whole apparatus. For each of the shaken and unshaken treatments, three bottles contained plant

tissue and an air bubble, three contained plant tissue only. The air bubbles in the static bottles were introduced as controls to illustrate any effect of the presence of a gas phase per se. It was assumed that if ^{14}C was lost to the gas phase as $^{14}CO_2$ this would result, if anything, in an underestimate of any apparent photosynthesis in bottles with bubbles. The experiment was performed in October using Porphyra at 9° C with an incubation time of 3.5 h. An irradiance of 15 μWcm^{-2} was used in order to ensure that the algal tissue was light saturated, and thus show up any limitation in carbon supply. The results are presented in Table 3.1. Each value is the mean of two replicates.

Table 3.1. Rates of photosynthesis in Porphyra measured under shaken and static conditions (^{14}C method)

$\mu gCcm^{-2}h^{-1}$

	Air bubble present	No air bubble
Shaken	10.42 ± 0.26	3.37 ± 0.65
Static	5.56 ± 0.29	4.61 ± 0.57

The rate observed for tissue incubated with an air bubble present and shaken was approximately twice the values recorded for the three other treatments. It seems probable that these three treatments did not differ significantly. It is presumed that the enhanced carbon uptake in the "shaken with bubble" treatment was due to the effectiveness of the turbulence produced by the moving bubble in breaking up boundary layers and

so reducing limitation of carbon supply. The "shaken without bubble" treatment actually produced the lowest result, indicating that no effective agitation could have been occurring. The possibility should not be overlooked that the constantly changing orientation of the discs in the "shaken with bubble" treatment may have been of extra advantage in light collection. It seems probable, however, that the converse could be equally true, since tissue in the unagitated bottles was constantly orientated towards the light.

These results confirmed the doubts raised by the coloured tracer experiment regarding the inefficacy of the horizontal shaker method with completely filled bottles, and led to the design of the tilting shaker described above. The implication of these results is that unshaken *in situ* experiments would give lower values than shaken laboratory experiments when both were performed under saturating irradiance. The question of whether shaken or unshaken results represent what occurs in nature is dealt with in the discussion of this chapter.

b. Respiration using tilting shaker (oxygen method)

No experiments were conducted to compare directly the rates of respiration obtained from shaken and static conditions. However, data obtained in the time-course study (see later this chapter) can be used to compare respiration rates obtained with these two treatments, albeit conducted in different seasons. Table 3.2 shows values of the steady state respiration rate achieved by *Porphyra* after incubation at 13°C for 1 to 5 h. Each value is the mean ± standard error of sixteen determinations.

Table 3.2. Rates of respiration in Porphyra measured under shaken and static conditions (oxygen method)

	$mgO_2g^{-1}h^{-1}$	Month	
Shaken	0.708 ± 0.029	June	(Figure 3.6D)
Static	0.750 ± 0.036	December	(Figure 3.6B)

These results are not significantly different and therefore tend to indicate either that shaking does not enhance the respiratory uptake of oxygen or that static conditions do not limit it. The difference in season, however, means that drawing this conclusion from these results alone is not entirely justified.

E. A. Drew (pers. comm.) has performed close comparisons of respiration using the tilting shaker with Laminaria digitata as experimental material. The measured respiratory rate was $0.3mgO_2g^{-1}h^{-1}$ and there was no significant difference between shaken and unshaken treatments. Photosynthesis measured concurrently, using the oxygen technique, showed a 2-fold enhancement when shaken.

4. The effect of current velocity on measured photosynthetic rate (^{14}C method)

In order to quantify to some extent the effect of water movement in a way not possible by shaking experiments, the effects of incubating tissue in different velocities of flowing water were studied. Four experiments

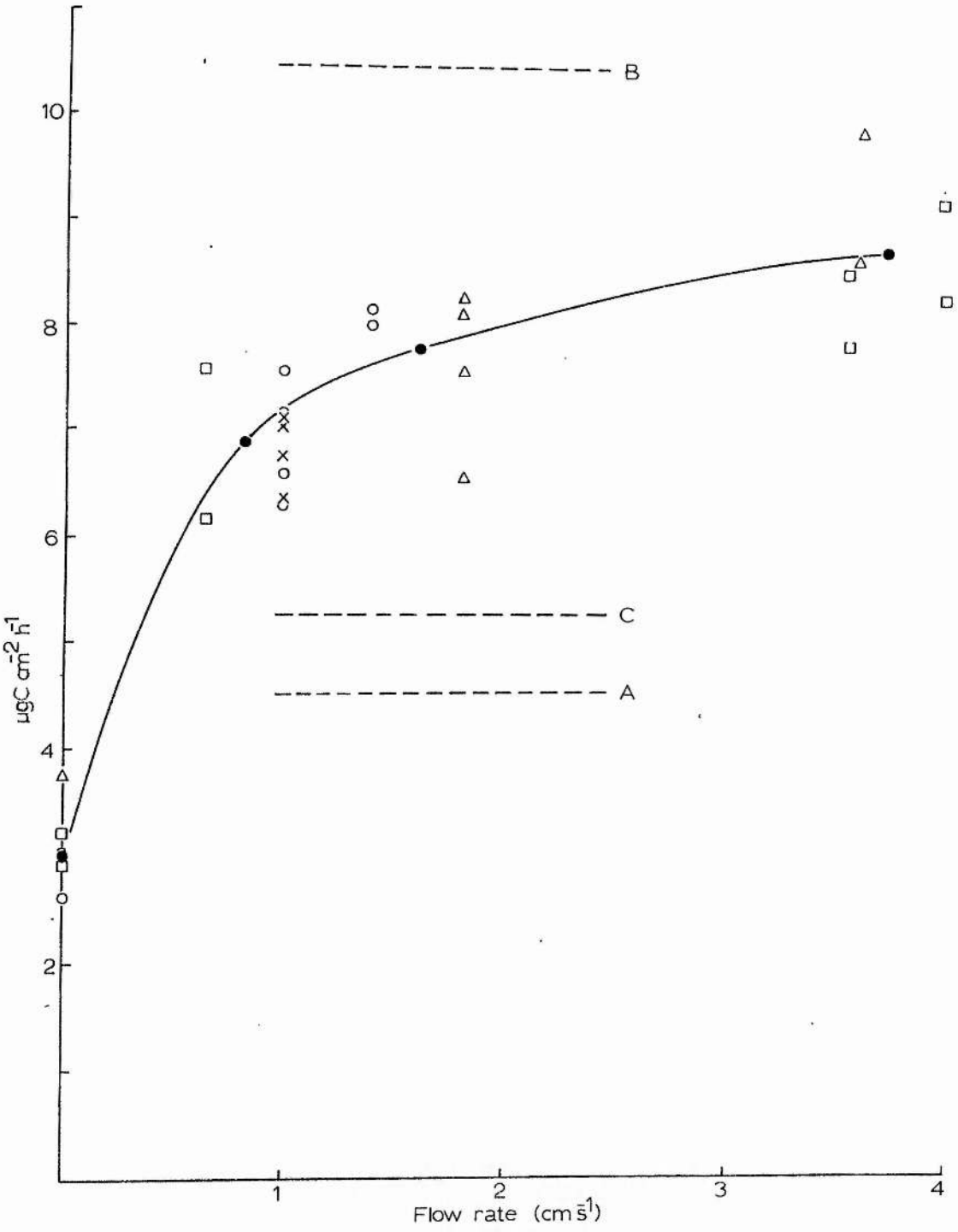


Figure 3.2. Effect of water flow velocity upon photosynthesis in Porphyra under fluorescent light source (curve fitted by eye), also: A, photosynthesis in unshaken conditions under tungsten light source; B, photosynthesis in shaken conditions under tungsten light source; C, photosynthesis in shaken conditions under fluorescent light source.

were conducted in May using the continuous flow apparatus and fluorescent light source described in Chapter 2. Samples of Porphyra of dimensions 6 x 0.75 cm (4.5 cm^2) were incubated for 2.5 h at 13°C. In each experiment at least two replicate pieces of tissue were incubated at each current velocity. It was not possible to replicate current velocities exactly in each experiment. The results are plotted in Figure 3.2. Mean values and standard errors were calculated for each "block" of results at current velocities of 0, 0.83, 1.61 and 3.74 cm s^{-1}. These mean values are significantly different and form a curve indicating that there is an enhancing effect of current velocity upon photosynthetic rate which is close to a maximum at 4 cm s^{-1}. The rate at this velocity is approximately two and a half times the static rate.

These experiments were carried out before the saturation intensity of Porphyra was ascertained (chapter 7), it is possible therefore that the low irradiance employed here (1.6 $mWcm^{-2}$) was limiting the photosynthetic rate at the higher current velocities. The rates at zero and maximum flow respectively are both below their shaken and unshaken counterparts in the tungsten light source experiment (irradiance 15 $mWcm^{-2}$, see Table 3.1 for rates) as shown by A and B in Figure 3.2. This means that the "saturation velocity" of 1 - 2 cm s^{-1} may be determined in the flow experiments by irradiance, and if irradiance were increased, a different relationship might develop between static and flow conditions.

The only other experiment performed using this fluorescent light source was done on Porphyra discs of area 4 cm^2 incubated in open vessels (using ^{14}C) shaken gently by an oscillating-table (4 to 5 times per min). The incubation was carried out in June at 15° C for 3 h. The mean photosynthetic rate of nine discs in three separate vessels was 5.25 ± 0,48 $\mu gCcm^{-2} h^{-1}$

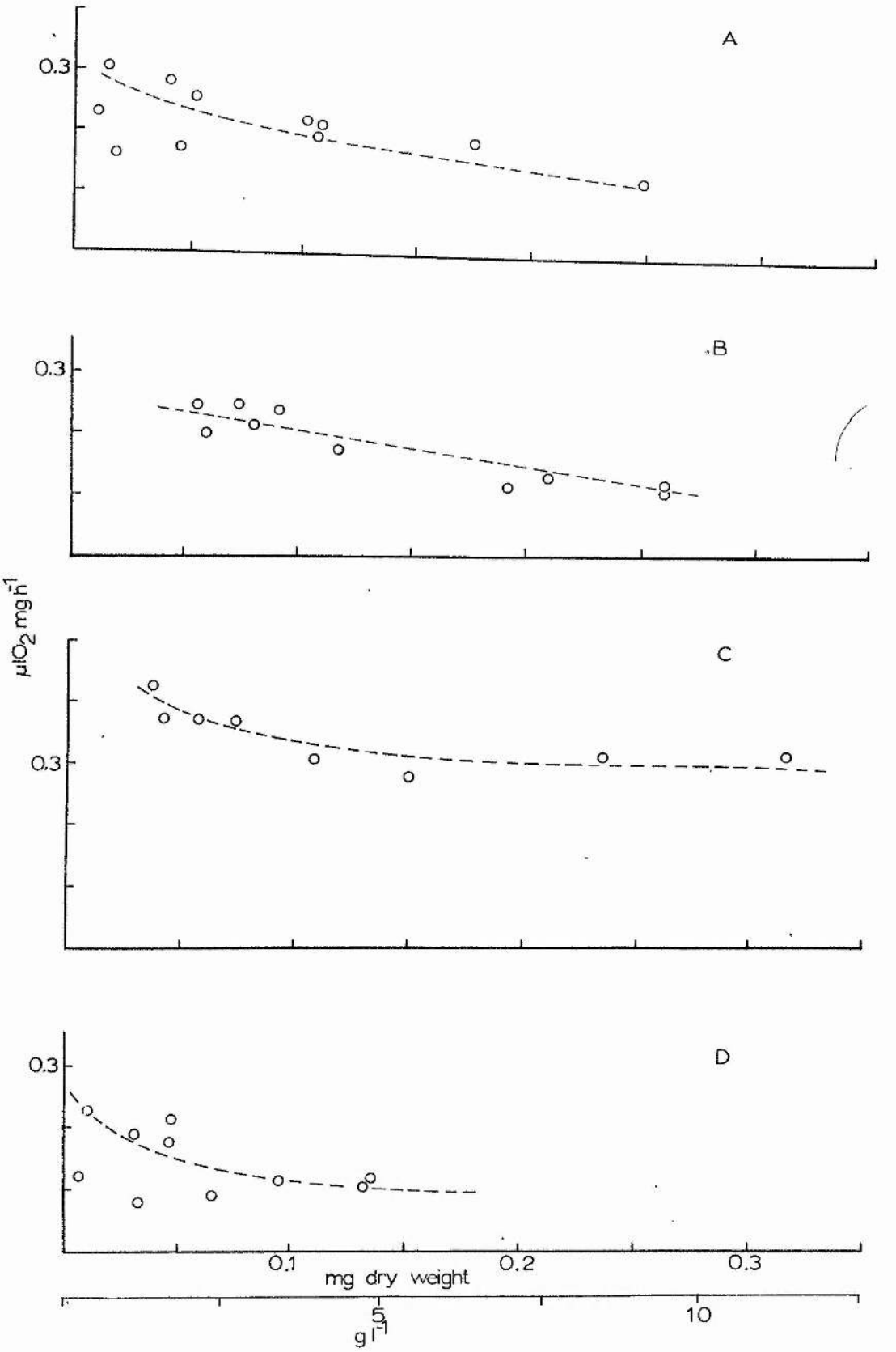
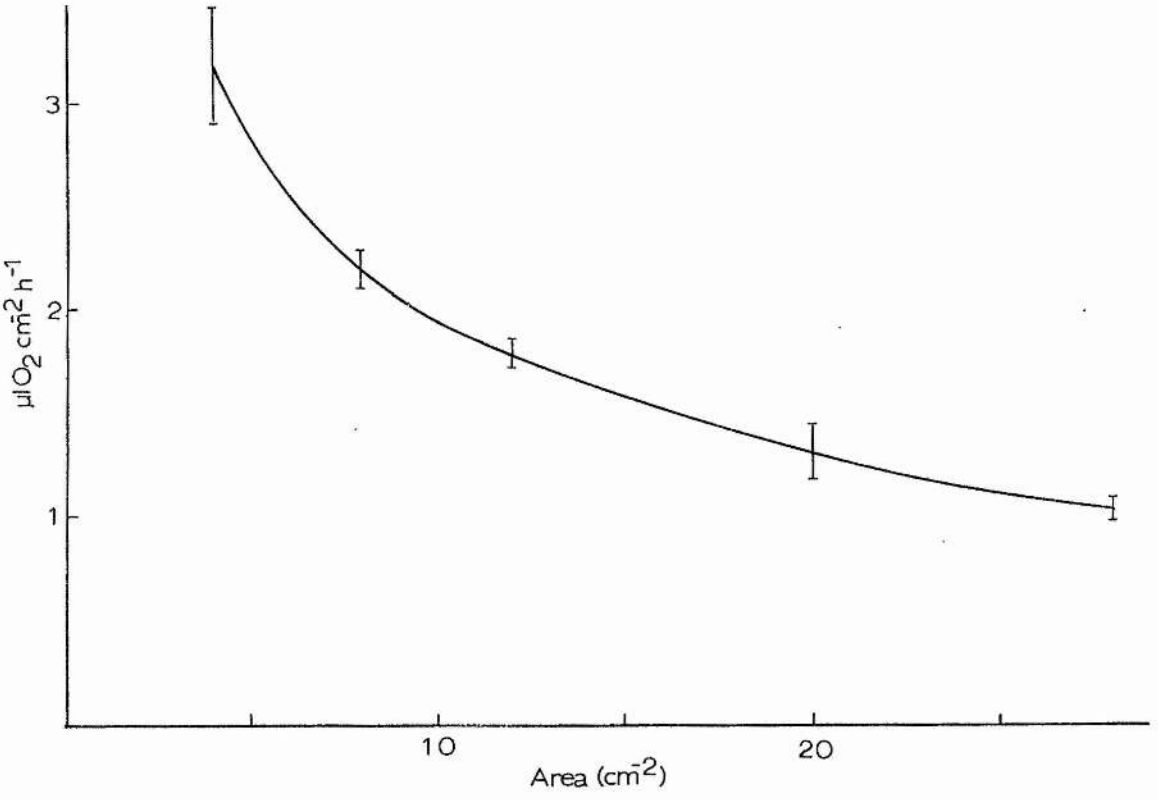


Figure 3.3. Relationship between measured rate of respiration and the ratio tissue-mass : incubation volume, A, Dumortia 7°C, 4h, r_{xy} = -0.59; B, Dilsea, 13°C, 3.5h, r_{xy} = -0.92; C, Laurencia, 9°C, 1h, r_{xy} = -0.65; D, Porphyra, 7°C, [illegible] r_{xy} = -0.44. Experiments conducted in laboratory in December under static [illegible] conditions. Scales on abscissa show mg dry weight per 28ml bottle and g dry [illegible] per litre.

which is shown at C in Figure 3.2. The effect of this gentle shaking is intermediate between the rates attained at zero and maximum flow and confirm that rates are indeed lower with this light source than the tungsten-iodide one.

5. The effect of the ratio of tissue-mass : incubation volume on the measured rate of respiration and photosynthesis (oxygen method)

Depletion in experimental vessels and in the sea is a function of the ratio between mass of matabolising organic matter and the volume of medium available for uptake. Five experiments were conducted using Laurencia, Dilsea, Dumontia and Porphyra. The incubation volume was maintained constant, using 28 ml incubation bottles, and varying amounts of algal tissue were selected by eye to cover a range from less than 0.01 to greater than 0.20 g of dry weight. In the cases of Porphyra, Laurencia and Dumontia, fragments of thallus were used, but with Dilsea, 4 cm^2 discs were cut and various numbers of these were mounted on brass needles (like a "spike-file") to keep the discs from resting on each other and thus limiting diffusion. The experiments were carried out in December at various temperatures and incubation times (see graphs) and under static conditions to duplicate as closely as possible the in situ treatment. The respiration rates, expressed per unit dry weight, are presented in Figures 3.3A, B, C and D. All the experiments showed a variability of values frequently found in measurement of respiratory rate (Chapter 8) but in general there was a slight downward



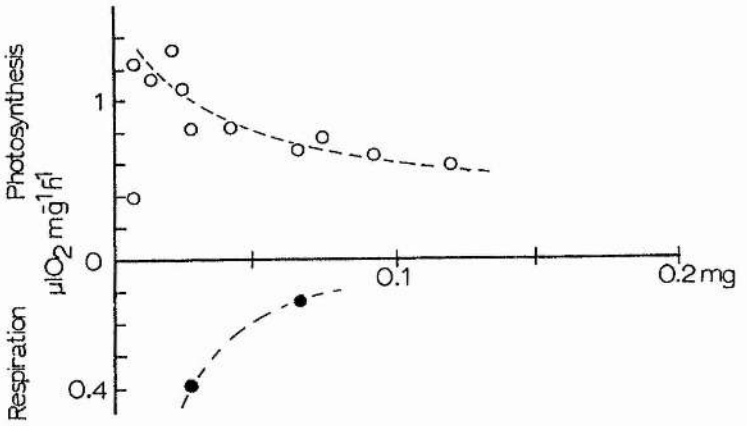


Figure 3.4. (upper). Relationship between measured rate of respiration (area basis) and thallus area present in 28ml incubation volume; Dilsea, December, 13°C, 3.5h, static, r_{xy} = -0.91 (c.f. same result on dry weight basis, Figure 3.3B).

Figure 3.5. (lower). Relationship between measured rates of both photosynthesis and respiration, and the ratio tissue-mass: incubation volume (28ml); Laurencia, December, 5°C, 3.5h, static, r_{xy} (photosynthesis) = -0.51.

trend of measured rate with increasing ratio of tissue : volume. Correlation coefficients (r_{xy}) calculated for each scatter diagram were negative in each case but only with Dilsea was the coefficient significant above the 5% level for the sample size concerned. In the case of Dilsea the coefficient was in fact above the 1% significance level. If the true relationship between tissue : volume ratio and measured respiration rate were not perfectly linear, then the correlation coefficient would not be strictly applicable here. This is a possible reason for the low significance of the coefficients.

Dilsea, incubated at the highest temperature used, showed the greatest and most significant decrease, of 50%, over a range from 1.7 - 8.7 gl^{-1}, suggesting that the tissue mass factor may well be more significant at higher temperatures. It is possible that the small decrease in rate shown by Laurencia was in part due to the short incubation time which could reduce the chance of depletion occurring even with quite high tissue masses. When plotted on an area basis (Figure 3.4) the Dilsea results show a more pronounced decrease, of about 67%, over the range 4 - 28 cm^2. This may be an artefact due to the low SLA value determined for the replicate discs at 4 cm^2. SLA of Dilsea has been shown to vary sequentially along the thallus (Figure 5.16) and it may be that the first two discs cut happened to be near the base of the plant, therefore thicker. It should be realised that, by its nature, dark respiration tends to increase SLA by depleting stored material.

The only experiment conducted on the effect of tissue mass : volume ratio on photosynthetic rate, (Figure 3.5) also showed a decrease in rate as the value of the ratio increased. The replicate samples used to measure dark respiration in this experiment showed a wide variation which may have been due to tissue : volume effects.

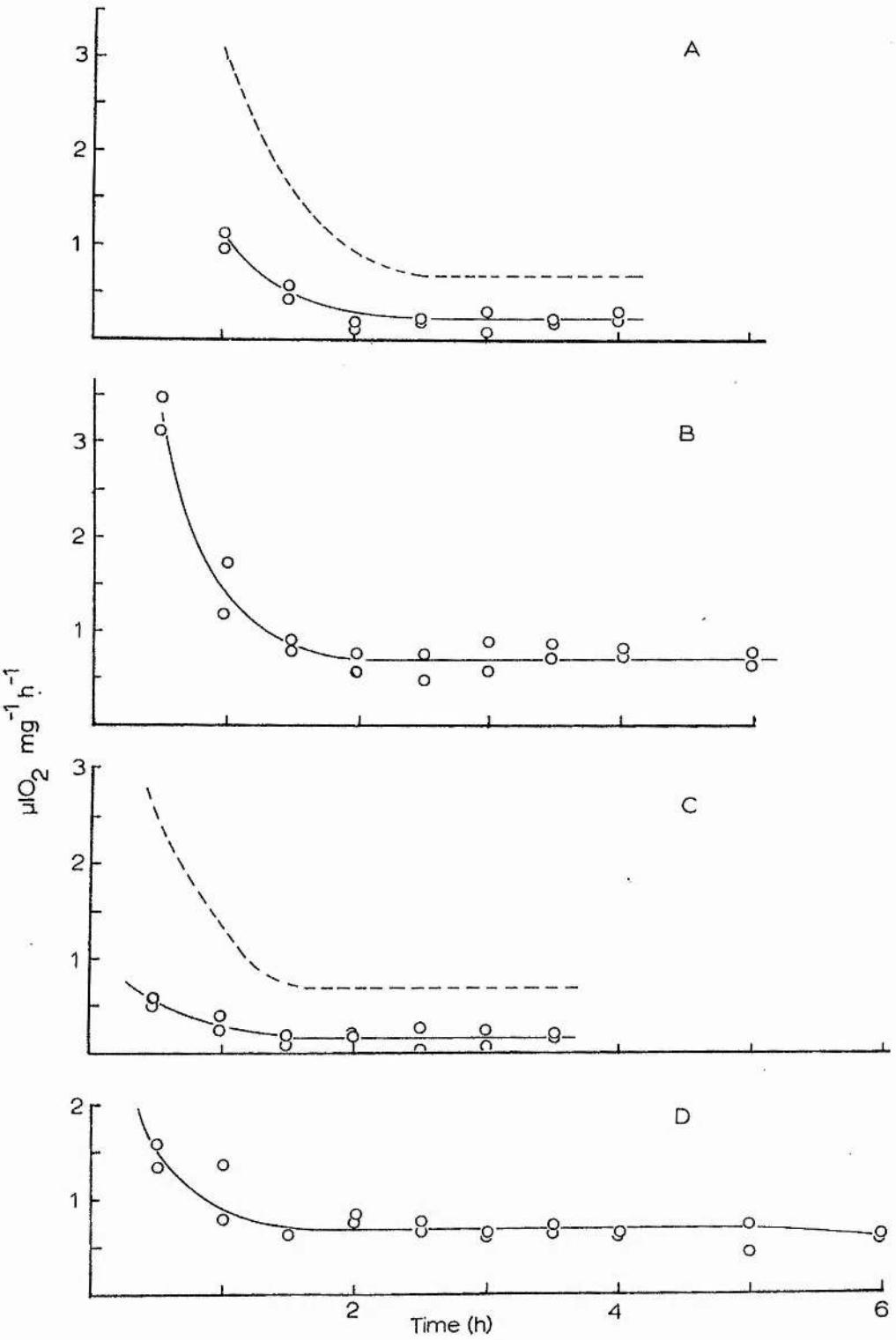


Figure 3.6. The relationship between measured rate of respiration and incubation time-course, A, Dumontia, December, 7°C, static; B, Porphyra, December, 13°C, static; C, Porphyra, December, 6.5°C, static; D, Porphyra, June, 13°C, shaken. Broken lines show curves normalised to steady-state rate of 0.7 µl O_2 mg^{-1} h^{-1}.

6. The effect of incubation time-course on measured rates of respiration (oxygen method)

Depletion in experimental vessels is a function not only of rate of uptake and volume of vessel but also time of incubation. This latter parameter was investigated in four experiments described below, three involving Porphyra and one, Dumontia. In each case, all the experimental bottles, containing the algal tissue, were set up together at the commencement of the experiment and pairs of replicates were removed for oxygen assay at intervals of ½ or 1 hour. The results, expressed as hourly rates of respiratory oxygen uptake, are plotted in Figure 3.6A, B, C and D together with data concerning temperature and month. Only one of the experiments was shaken (Figure 3.6D) the rest being static. The general pattern shows an initial high rate tailing off to a steady rate at 1.5 h in the case of Porphyra, 2 h in Dumontia. The low absolute rates shown in Figure 3.6C for statically incubated Porphyra may be due to the low temperature (6.5°C) compared with Figure 3.6B (13.0°C). In this connection, the steady state rate for Dumontia is probably not significantly different from that obtained for Porphyra at a similar temperature (cf. Figure 3.6A and C).

The results pose the questions (1) are the initial high rates the "true" respiratory rates, and the steady state a situation where the oxygen uptake is limited by the resistance to diffusion caused by a boundary layer of depleted oxygen content, or, (2) are the initial values artifactual, caused by a fault in the method, and the steady rate the "true" rate measured after the system has equilibrated? If the first suggestion holds, and the initial rates are closer to the "true" values, the "true" rates themselves may be yet higher, if measured between 0 and 0.5 h, beyond the limits of the sensitivity of the technique. To clarify this point, the

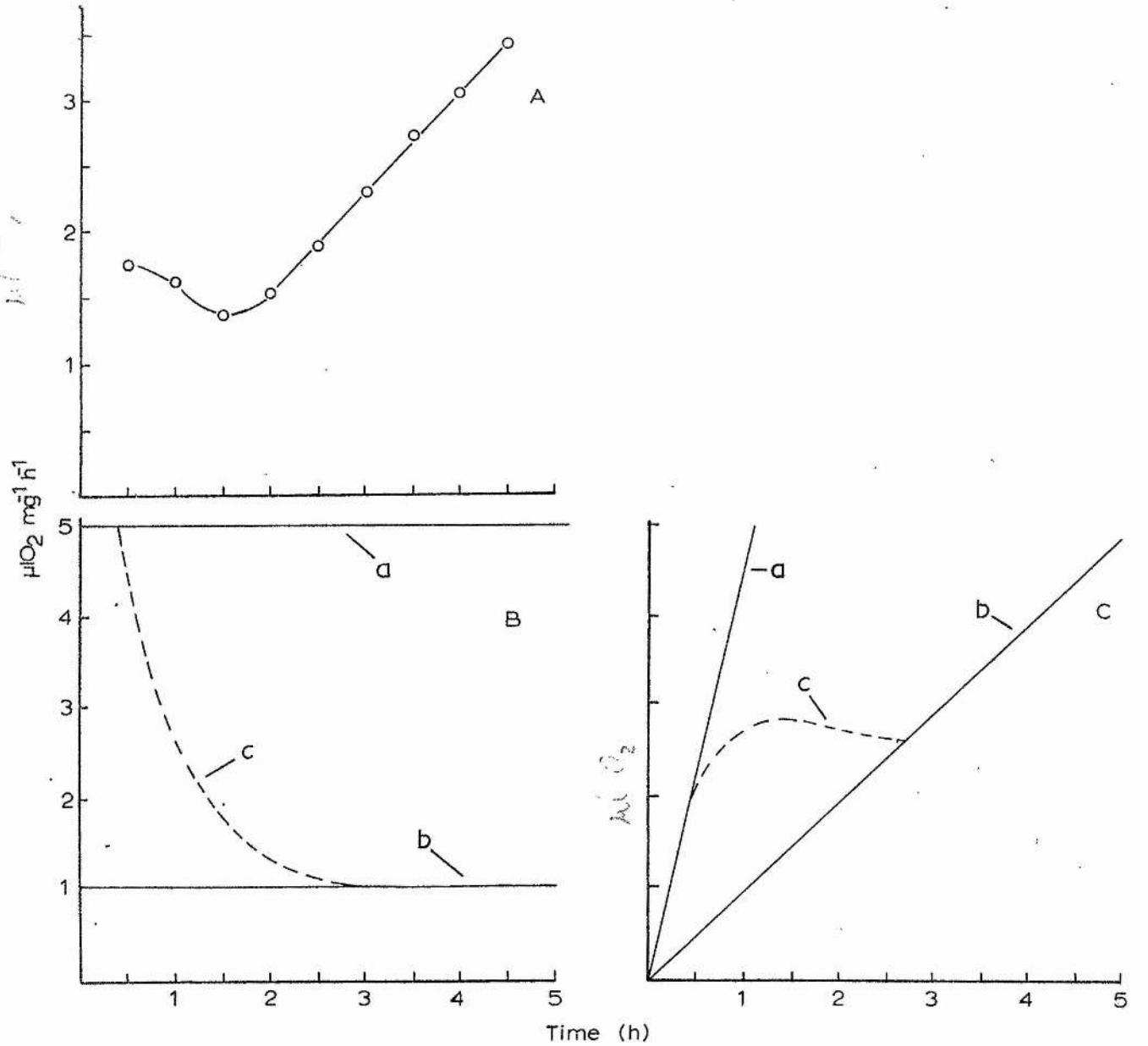


Figure 3.7. Theoretical explanation of Figure 3.6B; A, accumulated half-hourly oxygen uptake; B, high initial uptake rate (curve a), low-initial uptake rate (curve b), limitation of high initial rate (curve c); C, accumulated oxygen uptake re-plotted from B.

results from Figure 3.6B, the most extreme case, are re-plotted in Figure 3.7A as the accumulated half-hourly uptake of oxygen. The curve does not appear to pass through the origin, because the rate is not constant. The possibility that oxygen uptake occurs instantaneously at time zero, perhaps by some form of rapid oxidation can be ruled out from evidence from trials made by placing algal tissue in bottles and titrating immediately, when no departure from control oxygen content was shown. Figure 3.7B shows a theoretical explanation of Figure 3.6B. Curve (a) shows the situation if the initial high rate were the "true" uptake rate and (b) if the steady-state rate were the "true" rate, assuming both to be measured in a perfectly constant manner. Curve (c) shows a situation where the "true" rate is as curve (a), but due to limitation of oxygen due perhaps to formation of a boundary layer, the rate has been depressed to a secondary, apparently constant rate which can be supplied by diffusion. Figure 3.7C shows these curves re-plotted as accumulated oxygen uptake. Clearly curve (c) in Figure 3.7 B and C compares closely with the actual situation met with in Figures 3.6B and 3.7A, and indicates that the latter can be explained in terms of oxygen limitation. It is still possible however that even higher rates could be achieved by measurement earlier in the time-course than 0.5 h, and it is equally possible that the initial high rates are truly some form of transient phenomena, related, for instance, to tissue injury.

From Figure 3.7A, it appears that since the accumulated oxygen uptake at 1.5 h is actually less than it was at 1.0 and 0.5 h, the evolution of oxygen must have occurred. At these short intervals, experimental error is necessarily greater than over longer periods, but scrutiny of the oxygen content of the experimental bottles (Figure 3.8A; B, C and D) reveals

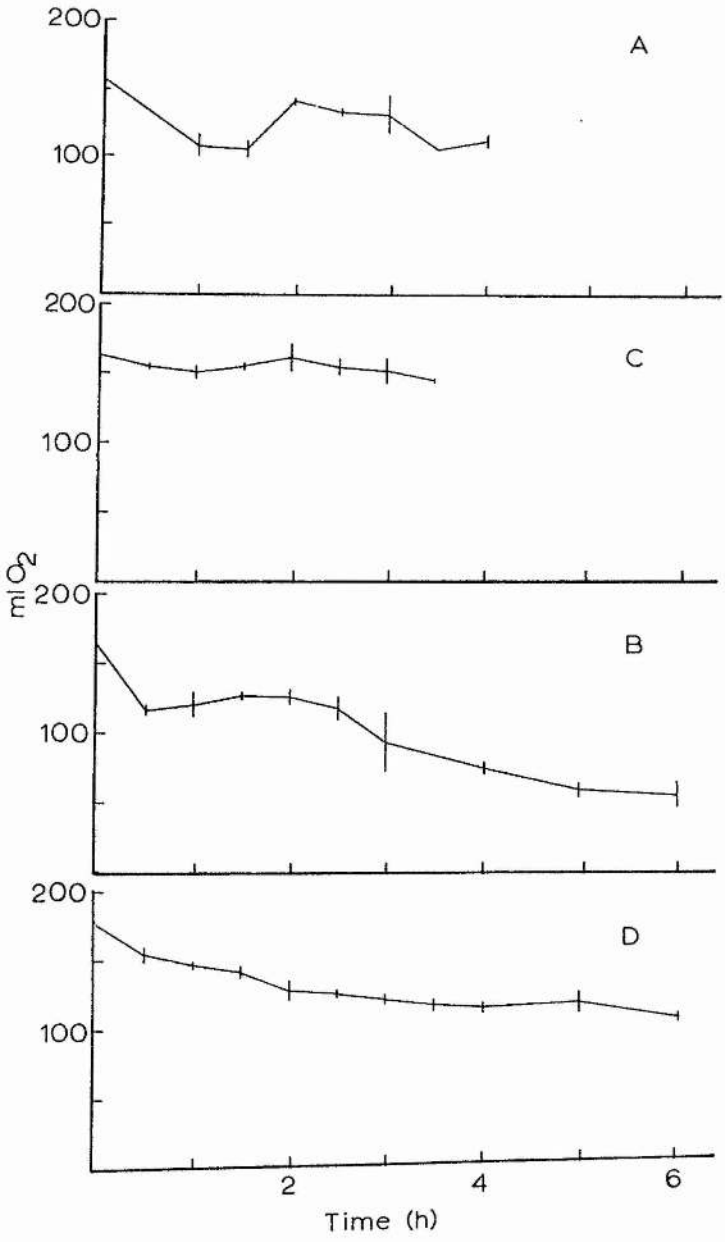


Figure 3.8. Progress of depletion of oxygen contents of incubation bottles used in experiments shown in Figure 3.6.

that there is a tendency for values after 1.5 to 2 h to be slightly higher than values at 0.5 to 1 h. This tendency is least apparent in the shaken experiment, Figure 3.8D. There is no obvious explanation for this occurrence and in the absence of more complete data no firm conclusion can be made. It seems more likely that as in Figure 3.7C, curve (c), the accumulated uptake would show a gradual transition from the initial rate to the steady-state rate.

Finally, although the above theoretical explanation does point to oxygen limitation as an explanation of the initial high rates and consequent "tail-off", if this were the case, the shaken experiment should have given a steady high rate from the start. Also, respiration rates are very low (compared for instance to rates of photosynthesis) and may well be able to be supplied by passive diffusion.

7. Discussion

a. Methodological considerations

As early as 1933, Emerson & Green had issued the caution that large tissue : volume ratios used in prior experiments by other workers could have produced unnaturally depleted situations when favourable light conditions produced high photosynthetic rates. They found that photosynthesis decreased from its initial rate after approximately 30 min using a tissue mass : volume ratio of 3.5 gl^{-1}. They decided that the initial high rate was the "true" rate becuase it was attained by, and remained constant in, algae incubated in seawater enriched with extra carbon source. Many

workers have attempted to reduce the effects of stagnation in field incubation experiments by allowing bottles to be shaken by wave movement (Sargent & Lantrip 1952; Johnston & Cook 1968; Jupp 1972; Drew 1973). As has been shown above, however, shaking of completely water-filled vessels may not ensure effective mixing. To overcome this, Sargent & Lantrip (1952) in field experiments on Macrocystis, and Tseng & Sweeney (1946) in shaken laboratory experiments on Gelidium enclosed two glass marbles in the incubation bottles to act as stirrers. Care would be required to ensure that tissue damage did not occur to delicate species using this practice. Wetzel (1973) recommended the use of battery-operated stirrers when incubating marine algae in perspex cylinders embedded (therefore necessarily static) in the substrate. Johnston & Cook (1968) recommended very low tissue : volume ratios of around 0.3 gl^{-1} but this was related to the extremely long time-course (24 h) of their experiments. In the present study, a 27 h time-course experiment involved a tissue:volume ratio of 0.16 gl^{-1} in 450 ml bottles and yielded results essentially similar to those obtained in short-term (4 h) experiments using 1.2 gl^{-1} in 30 ml bottles. Tseng & Sweeney (1946) concluded that even the work of Emerson & Green (1933) was suspect due to high tissue : volume ratios, and found experimentally that 1.27 gl^{-1} in a 550 ml vessel produced a limiting situation whilst 0.74 gl^{-1} in 270 ml did not. Tschudy (1934) in early in situ experiments incubated samples of algae for up to 10 h with a tissue : volume ratio of 2 to 10 gl^{-1} (in 80 ml vessels) thus almost certainly producing a limiting situation (see also Table 6.11), since the measured rates were extremely low.

In respect of water movement in experimental conditions and its relation to the natural state, Doty & Oguri (1958) found an increase of about 50% in phytoplankton fixation using various modes of agitation but

expressed doubts as to whether stagnant or shaken conditions reflected the natural situation most. In work on macrophytes, Kain et al.(1975) stressed the difficulty of exact simulation of appropriate water movement in in situ experiments and emphasised the effect that lack of this may have on the interpretation of relationships between calculated compensation depth and observed lower limits of growth. Nath (cited in Schwenke 1971) found that in manometric studies of oxygen uptake by Fucus serratus, shaking produced a 2-fold increase over static conditions, and also found that frequency and amplitude of shaking were important in the enhancement effect. Finally, Gessner & Pannier (1958) found enhanced respiratory uptake of oxygen in phytoplankton in shaken Warburg flasks compared with unshaken Winkler bottles, but concluded "Obviously only the Winkler method approaches natural conditions because even in stormy weather plankton cells surely are not shaken 100 times a minute as in the Warburg vessels". At present there is little evidence on which to base such conclusions but it may be said that measurements of photosynthesis and respiration made under static conditions probably always underestimate the natural case and stirring is a desirable feature in experimental work. However, the degree of water movement required by different species is not known and awaits accurate in situ measurement of water movements habitually acting on littoral and sublittoral algae.

Due to the relatively small volume (28 ml) of the incubation vessels used in the present work, a significant depletion of the total available oxygen and carbon source was inevitable, the measurement of this depletion in fact forming the basis of the oxygen method. Table 3.3 shows a range of maximal photosynthetic and respiration rates found by the present methods, and the extent of depletion engendered by them.

Table 3.3 Maximum possible extent of depletion of carbon and oxygen in experimental vessels under different conditions

Conditions	Species	Photo-synthesis $\mu gC cm^{-2} h^{-1}$	Tissue Area cm^2	Time h	Total Uptake µgC	Carbon Available µgC	% C Removed
Britain laboratory	Porphyra	25	4	1	100	780	12.8
Britain in situ	Porphyra	6	8	3	144	780	18.5
Britain in situ	Rhodymenia	12	8	4.3	413	780	52.9
Sicily in situ	Ulva	4	4	6	96	900	10.7

Conditions	Species	Respira-tion $\mu l O_2 mg^{-1} h^{-1}$	Tissue dry weight mg	Time h	Total Uptake $\mu l O_2$	Oxygen Available $\mu l O_2$	% O_2 Removed
Britain in situ	Porphyra	0.352	26.7	4	37.6	178	21.1
Sicily in situ	Peyssonelia	0.220	70.0	8	123.0	160	76.9

As these are maximal values, it can be seen that photosynthesis rarely utilised more than 50% of available carbon, and in the vast majority of cases, especially in deep in situ incubations where rates were low due to low irradiance, used 12% or less. In respiration experiments, depletion was more marked because the concentration of oxygen was lower than that of carbon, but the relatively low respiration rates partially off-set this.

It is probable that all the British incubations were below the maximum of 20% shown in the Table 3.4. At Ganzirri, the long incubation periods necessitated by the hydrographic conditions (Chapter 5) occasionally resulted in depletions, as in the case of Peyssonelia, which were higher than desired. However, most respiration rates were approximately half that for Peyssonelia and, in fact, only rarely did the oxygen content of the incubation bottles fall below half of the control value.

b. Physiological considerations

The principal physiological effect of water movement is to reduce the effective thickness of the boundary layer, thereby increasing the availability of solutes. In view of this, any differential effect of water movement on the processes of respiration and photosynthesis must be due to the mobility or concentration of the ionic species concerned. The diffusivities of O_2, CO_2 and HCO_3^- in water are very similar, the values for their diffusion coefficients being 0.29×10^{-4}, 0.16×10^{-4} and 0.14×10^{-4} $cm^2 s^{-1}$ respectively (Šesták et al. 1971). The concentration of bicarbonate however, is about 8 times that of oxygen in natural seawater and this would, if anything, result in a greater effect of water movement upon respiration than photosynthesis (assuming bicarbonate, not carbon dioxide, to be the carbon source of photosynthesis). Westlake (1967) found respiration in freshwater macrophytes was "scarcely" affected by water flow if sufficient oxygen was present (i.e. in equilibrium with air), but found that the photosynthetic rate increased to a saturation point, by a factor of 6 (c.f. 2.5 in present study Figure 3.2) in a flow rate of 0.5 cm s^{-1} compared with static rates. This differential effect is consistent with the limited results on this aspect in the present work, and of Drew (pers. comm.). Most workers, however, report that water agitation and flow

have a similar effect upon photosynthesis and respiration. Steemann-Nielsen (1942) found an approximately 2-fold increase in photosynthesis in Fucus serratus when shaken and respiration to be enhanced to only a slightly smaller extent. Gessner (1940, 1955) and Printz (cited in Gessner 1955) found respiration and photosynthesis of several marine algae and freshwater macrophytes to be enhanced by stirring, up to 2 times the static rates. Although in individual species, stirring frequently had a differential effect on the two processes, the pattern was not consistent. The relatively low enhancement of photosynthesis might have been due to low irradiances (unspecified) used in these experiments.

On a different order of magnitude from the findings of Westlake (1967) and the present study, Conover (1967) found the daily net photosynthesis of Zostera sp. to increase linearly with current velocity up to 40 cm s^{-1}, and emphasised the role of water currents in continually presenting nutrients at the seawater/membrane interface. Whitford (1960) stated a 15 cm s^{-1} threshold for any effect of current on growth to be manifest in freshwater algae, a figure greater by 30 times the threshold for detectable increase in carbon fixation measured in the present work. In studies of respiration in freshwater filamentous algae, Whitford (1960) and Whitford & Schumacher (1961) found that respiration rate was more enhanced by current in lotic (flowing water) adapted algae than lenitic (stagnant water) species. It is possible that this sort of adaptation could occur in the sea where deep-growing species are rarely exposed to the high current velocities experienced by intertidal algae. In this connection, Gessner (1940, 1955) in a study of sixteen marine macroalgal species, found that respiration of the sublittoral forms was significantly less enhanced by stirring of the medium than in littoral species, and concluded this to indicate adaptation to low current velocities prevailing at depth. Schwenke (1971) attributed the survival of the sublittoral species Delesseria sanguinea and Phycodrys rubens for months in stagnating

water in the Baltic Sea with an ability to adapt to low supply conditions.

Schumacher & Whitford (1965) found respiration in the freshwater red algae Batrachospermum macrosporum and Audouinella violacea to be enhanced 1.6 and 7-fold respectively when in a current velocity of 18 cm s^{-1} as opposed to static conditions. The uptake of phosphate using P^{32} tracer was found to be similarly related to current velocity. More importantly, these authors found that algae free-floating in a current of 18 cm s^{-1} did not show as much phosphate uptake enhancement as algae attached in the current, demonstrating the importance of the "sweeping" effect of water flow on attached algae. Matsumoto (1969) found that enriching the sea water with nitrate and phosphate reduced the optimal water velocity for growth of "Nori" (Porphyra tenera) from 20 cm s^{-1} to 15 cm s^{-1} but dilution of seawater with unenriched saline solution raised the optimal velocity to 30 cm s^{-1}, showing the role of currents in modifying the effective concentration of solutes available to algae.

In an empirical study of boundary layer formation around excised barley roots and discs of potato and beet, Polle & Jenny (1971) found that uptake of rubidium was strongly controlled by the rate of stirring of the bathing medium. Using a modification of the Nernst equation they calculated boundary layer thicknesses of the order of 30 μm minimum at the highest stirring rate (25 cm s^{-1}) to 117 μm at the lowest rate (6.5 cm s^{-1}). Their equation was in the form:

$$\delta N = \frac{SDC_0 t}{M} \qquad (3.1)$$

where,

δ = thickness of Nernst boundary layer (cm)

S = surface area under consideration (cm^2)

C_0 = concentration of solute in stirred bulk solution (g cm^{-3})

D/

D = diffusion coefficient of the solute ($cm^2 s^{-1}$)

t = time period (s)

M = uptake of solute ($gcm^{-2} s^{-1}$)

Using equation 3.1, Nernst boundary layer thicknesses were computed for photosynthetic carbon uptake in the flow experiments on Porphyra (Figure 3.2) and for the maximum light-saturated photosynthetic rate attained by this species (Chapter 7, Figure 7). The values are presented in Table 3.4

Table 3.4 Nernst boundary layer thickness determined by method of Polle & Jenny (1971) for Porphyra photosynthetic carbon uptake

Current velocity	Concentration of carbon or bicarbonate	Photosynthesis	Layer thickness δN
$cm\ s^{-1}$	mM	$\mu gC cm^{-2} h^{-1}$	μm
0	2.17	3	4.4
4	2.17	9	1.5
Shaken	2.17	18	0.7

As can be seen, the boundary layer thicknesses obtained by this method are an order of magnitude smaller than those found by Polle & Jenny (1971) themselves. There are three possible reasons for this, firstly that the concentration of solute used here (2.17 mM) was much greater than in the experiments of Polle & Jenny (0.1 mM). Secondly, these workers emphasised the importance of edge-distance in modifying boundary

layer thickness and whilst their experiments involved the use of discs, with a relatively large edge distance, the present study (except the shaken experiment) utilised narrow strips of thallus with a correspondingly small edge distance. The third possibility is that solute transport was occurring not by molecular diffusion but by the much faster process of eddy diffusion which would render the use of D, the molecular diffusion coefficient inappropriate. Eddy diffusion coefficients are highly variable but always of greater magnitude than molecular coefficients and this would raise the value of the numerator in equation 3.1 resulting in a corresponding increase in the estimate of the thickness of the boundary layer. Raven (1970) suggests an unstirred layer of 100 μm or more around aquatic macroalgae, depending upon the degree of stirring. In addition to the unstirred layer there is a diffusion path within the plant material itself, through and between the cells and cell walls to the chloroplast, which has been estimated at 40 μm for submerged leaves of aquatic phanerogams (Steemann-Nielsen 1960). From observation under the light microscope of sections of Dilsea, the most massive alga investigated in the present study, the internal path length to the chloroplasts was estimated at 10 to 120 μm, the depth of the pigmented layer beneath the cuticle. In most species studied however, e.g. Porphyra, Delesseria, Ulva, which have total thallus thicknesses of less than 100 μm (Smith 1955; Föyn 1955) the internal path length is liable to be much smaller. There is no evidence in the present work to suggest that the effect of water movement on metabolism is greater in Dilsea than in the less massive species.

Raven (1970) points out that for plants unable to use bicarbonate directly, (i.e. they must use carbon dioxide) rapid water flow may be important in effectively increasing the availability of carbon dioxide to

them when the pH is high and the carbon dioxide tension correspondingly low. At normal pH in the sea (around 8.2) the concentration of bicarbonate is nearly 100 times that of carbon dioxide (Steeman-Nielsen 1975). Blinks (1963) and Joliffe & Tregunna (1970) showed that some marine macroalgae appear to be unable to use bicarbonate as their sole carbon source, for example Porphyra. Water movement may be of more importance to these species than to bicarbonate users, and they may tend to colonise areas of greater turbulence accordingly. Joliffe & Tregunna (1970) however cited only one other carbon dioxide user, Desmarestia munda, which is habitaully a deep water species, and therefore not subject to particularly great water movement. It also seems possible that if a bicarbonate user and not Porphyra, had been used in the agitation and water flow experiments, enhancement of photosynthesis might have been less marked. Raven (1970) has affirmed that the rate of photosynthesis under natural conditions can be limited by the rate of entry of inorganic carbon into the cell. Carbon dioxide diffuses readily across cell membranes whilst bicarbonate probably requires active transport to support measured photosynthetic rates (Raven 1974). It is further postulated that in algae which cannot utilise bicarbonate directly, the enzyme carbonic anhydrase may be effective in catalyzing the reversible dehydration of bicarbonate rendering carbon dioxide available within the cell (Raven 1974). Carbonic anhydrase has been reported in several marine algae including Ulva pertusa (green) and Serraticardia maxima (red) (Okazaki & Furuya 1971). Enns (1967) postulated that if carbonic anhydrase was present in cell membranes, it could effectively increase active bicarbonate uptake in solutions where this ion was the predominant carbon source, as in seawater. Clearly, further research is required into the carbon sources and uptake mechanisms of individual algal species before informed interpretations can be made

regarding their reactions to water movement.

In one of the few theoretical studies of the effects of water flow on uptake of solutes by aquatic plants, Munk & Riley (1952) discarded molecular diffusion as a means of nutrient supply to algae growing in the form of an attached flat plate of cells, and stated that supply of nutrients in any but extremely low current velocities would be by the process of forced convection. (This process is usually used to describe heat loss or gain from a surface situated in a current of fluid of different temperature. The exchange occurs through the boundary layer at a rate proportional to the velocity of the flow. Forced convection is exemplified by a household fan heater compared with the process of free convection as occurs in a normal convector heater - Monteith 1973.) Munk & Riley (1952) derived the following equation for maximum possible absorption rates by an attached plate:

$$v = \left(\frac{2q}{s}\right)^2 \cdot \frac{1}{\phi^2} \cdot \frac{d}{k'} \qquad (3.2)$$

which, rearranged gives:

$$q = \sqrt{\frac{vs^2\phi^2k'}{4d}} \qquad (3.3)$$

Where,

- v = current velocity (cm^2s^{-1})
- q = uptake of solute ($g\ cm^{-2}\ s^{-1}$)
- s = surface area (cm^2)
- ϕ = concentration of solute at a large distance from plant ($g\ cm^{-3}$)
- d = dimension in direction of flow (cm)
- k' = a function of k, molecular diffusion coefficient of the solute = $4.5 \times 10^{-6}\ cm^2s^{-1}$ for CO_2, HCO_3^- and O_2 (This is a complex function involving the Prandtl number.)

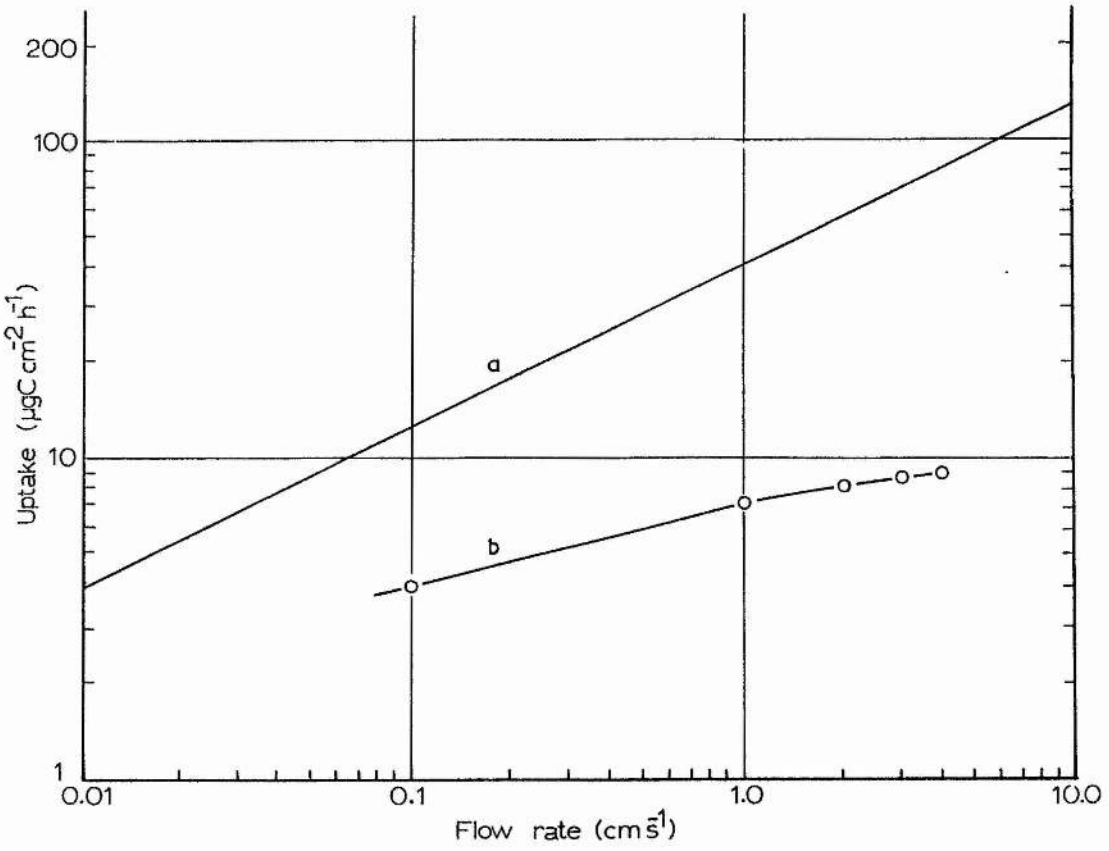


Figure 3.9. Relationship between uptake rate and flow rate; upper curve, as suggested by the forced convection hypothesis of Munk & Riley (1951); lower curve, actual carbon uptake rates observed in Porphyra (Figure 3.2).

Taking ϕ = 26 µg C cm^{-3} and d = 6 cm as used in the flow experiments (Figure 3.2) and using a series of values of v, a corresponding series of theoretically possible uptake rates of carbon can be produced, which is plotted in Figure 3.9 curve (a). From the figure it is seen that a flow rate of 4 cm s^{-1} as used in the experiments (Figure 3.2) could supply about 80 µg C cm^{-2} h^{-1}, compared with actual uptake of 9 µg C cm^{-2} h^{-1} (Figure 3.2 and 3.9 curve (b)) which this system could apparently still supply at a flow rate of 1 or even 0.1 cm s^{-1}. It is therefore not clear why photosynthesis of Porphyra should be limited by water flow at all, above velocities of 0.001 to 0.01 cm s^{-1} as used in the experiments. Two principal reasons why Munk & Riley's theoretical treatment is not borne out by experiment are that either it does not adequately model the situation under consideration, or another limiting factor is operating. Considering the first reason it should be said that Munk & Riley do not clearly indicate how the process of forced convection overcomes the resistance of boundary layers formed at very low current velocities. Secondly, it is possible, as has already been stated, that the levelling off of the curve in Figure 3.2 and 3.9 (curve b) may be due to light limitation, in which case, the rate under static and very low rates may also be a combination of limitation by light and carbon supply. The possibility of limiting factors other than those of light and inorganic carbon must not be overlooked.

c. Ecological significance of water movement and nutrient limitation

The marine littoral zone is an area of extreme water movement where surge velocities up to 1400 cm s^{-1} have been reported (Jones & Demetropolous 1968), equivalent to dynamic pressures of 1 to 1.5 kg cm^{-2} produced by waves

of 8 m height. The depth of action of waves is a function of their wavelength and amplitude. In the open ocean, water movement is caused by waves consists of a vertical series of orbitals of which the diameters decrease exponentially with increasing depth. In shallow water however, where waves "feel bottom" these orbitals become depressed into ellipses as the bottom is approached and at the bottom itself these ellipses have no vertical components at all, the motion consisting entirely of a horizontal to-and-fro surge (Carstens 1968; Neushel 1972). Calculations from equations of Carstens (1968) indicate that waves of height 1 m and length 25 m produce maximum bottom velocities of 150 cm s^{-1} at a depth of 2 m but only 1 cm s^{-1} at 20 m. Clearly, during calm weather, bottom velocities in the sublittoral zone can be extremely small, increasing the significance of molecular diffusive processes. Also, it is probable that the calculated effect of wave action upon water movement at depth is greatly reduced by drag factors operating in algal stands. For example, Ott (1967) found that a current velocity of 20 cm s^{-1} above a Sargassum canopy was reduced to 0.5 cm s^{-1} within it. Neushel (1972) defined four separate zones of water movement affecting marine macroalgae. These were (1)"current zone" layers occurring at distances greater than 2 to 3 m from the substratum and involving unidirectional currents. (2) "surge zone" occurring within 2 m of the substratum and where to-and-fro movements of the order of 100 cm s^{-1} predominate. (3) "boundary layer" (turbulent to laminar) in and among the smaller macrophytes, of thickness up to 1 cm, and involving water movements up to 10 cm s^{-1} (4) "boundary layer" (laminar sub-layer) of thickness up to 0.01 cm (100 µm) and maximum flow rates of 1 cm s^{-1}. Also, this worker made a differentiation between the mechanical, current boundary layer described (4) above, and the diffusion or "concentration gradient" boundary layer which

he stated was approximately one eighth of the thickness of the former. That algae can respond to extremely small flow velocities was shown by Seitz (1972) who found that Fucus eggs, which anchor within the laminar sub-layer of the boundary layer, can produce rhizoids which show a positive "rheotropic" response by growing upstream in a current of only 1×10^{-4} cm s^{-1} (i.e. 1 μm s^{-1}).

The zonation of algal species in response to varying degrees of exposure to wave action is well known (Lewis 1964, 1968; Jones & Demetropolous 1968; Schwenke 1971). Doty (1971) suggested that, in addition to physical damage to the thallus, calm water species may be "leached" by excessive outward diffusion of metabolites when exposed to conditions of high turbulence (a possibility also considered by Munk & Riley 1952, as the converse of their forced convection uptake hypothesis), and that conversely the massive laminarians do not thrive in conditions where large diffusion gradients of nutrients exist, in calm water. The thin undivided frond form adopted by laminarian species when growing in calm water (dealt with in more detail in Chapter 5), may enhance nutrient supply by reducing internal diffusion paths. Neushel (1972) suggested that the perforations, bullations and ribs found in macroalgal frond structure may be important in causing turbulent flow over their surfaces and thus favouring efficient nutrient supply. Charters et al. (1969) calculated that the morphology of Eisenia arborea was constructed so that its form was modified, in periods of strong current flow, to present the least possible hydrodynamic form drag, but during periods of calm it retained a shape which exposed maximum surface area for light collection. Similarly, in some of the few experiments conducted on this

topic in the field, Jones (1959) found that there was no effect of a current of 50 cm s^{-1} on growth of plants of Gracilaria verrucosa but growth was increased indirectly because the plants were streamed out horizontally by the current and were thus irradiated more efficiently.

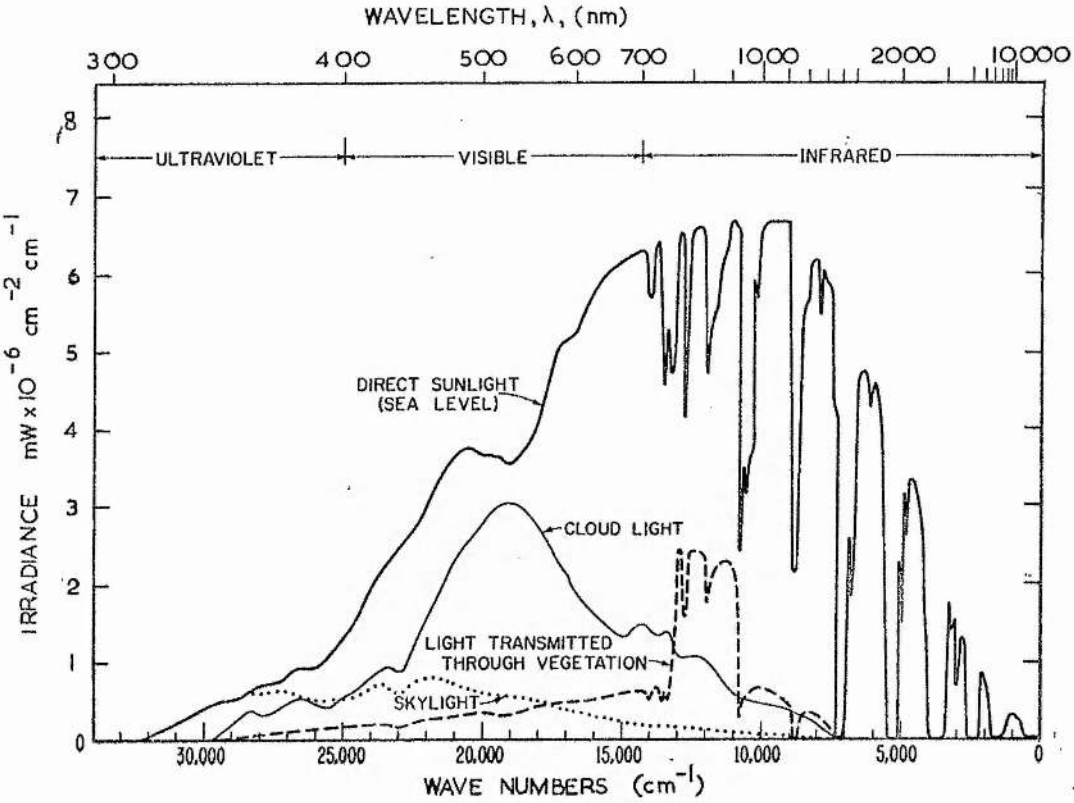
It is known that supply of oxygen can limit growth of organisms in nature in poorly stirred freshwater environments (Sculthorpe 1967) but it is generally considered that the marine environment is sufficiently well stirred to obviate such limitation (e.g. Raven 1970; Riedl 1971). Smith & Marsh (1973) have shown however, that the size of the standing crop of marine plants and animals living close to and within the surface of coral reef-flat pavements can be limited by diffusive supply of oxygen, and such a situation may well occur in dense kelp stands in calm water, at night, in temperate regions, albeit only for short periods. The high concentration of bicarbonate in seawater (about 8 times that of oxygen) would seem to indicate that limitation of photosynthesis by diffusive supply of inorganic carbon in the sea is less likely.

A theoretical consideration of diffusive supply and possible solute uptake is presented in Chapter 9.

CHAPTER 4

Light Availability

CONTENTS



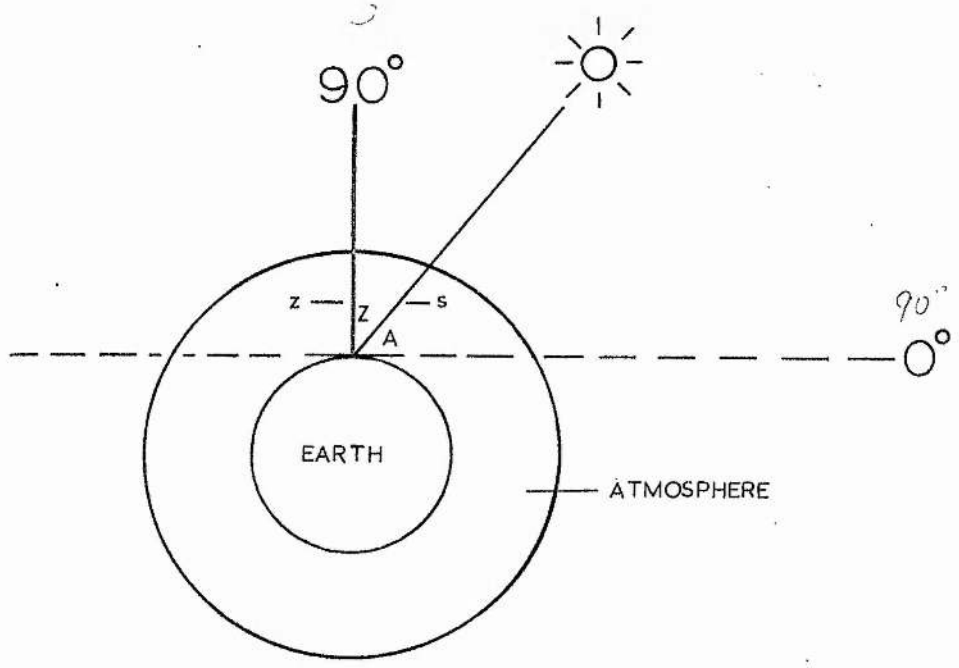


Figure 4.1. (upper). Spectral distribution of solar radiation at the Earth as a function of frequency (expressed in wave numbers, or reciprocal of wavelength in cm). Wavelength (λ) in nanometres given at top (from Gates 1965).

Figure 4.2. (lower). "Optical air mass" is equal to $\frac{z}{s}$, or secant Z; A is the altitude of the sun.

1. Introduction

The energy source used by photosynthetic organisms is contained in a small section of the electromagnetic spectrum and in the natural situation is provided by the sun. The energy can be described in terms both of particles and waves, and the wavelengths of radiations can be used to characterise the well known subdivisions of the solar spectrum (Figure 4.1). The spectrum available to plants on earth is greatly modified by the separate processes of selective absorption and scattering of different wavelengths during its passage through earth's atmosphere, and perhaps additionally by passage through plant canopies and, as in the present situation, through seawater. Although the sun emits radiation of wavelengths less than 10Å to greater than 100m, 98% of the energy is between 250 - 3000 nm (Robinson 1966) which includes the popularly-named light types, the ultraviolet, visible and infrared wavebands. Ultraviolet (UV) of wavelengths less than 280 nm is totally absrobed by the ozone layer at the top of the atmosphere. As a body, the earth absorbs the available radiation between 280 nm and 3000 nm and constantly re-radiates wavelengths from 3000nm to 100,000 nm. Radiation between approximately 800 and 100,000 nm is termed heat or infrared (IR) and can be absorbed and detected by the skin in man. Radiation from 400 - 700 nm is collected and perceived by the human eye, with a maximum sensitivity at 555 nm and is termed "light" or "visible light". This region of the spectrum also contains almost all the radiation absorbed by chlorophyll (with absorption peaks *in vivo* at 440 nm and 675 nm) and other pigments of photosynthetic plants and in this context is

termed "photosynthetically active radiation" or PAR. Due to differing pigment constitutions, the range of PAR is different for different plant species, but extends below 400 nm and above 700 nm in many cases (Rabinowitch 1956; Halldal 1967). Most active use however, seems to be between these limits and they provide a useful range for general purposes (Monteith 1973) used by many authors. Due to changes in the character of the atmosphere, the spectral composition of irradiance striking the ground is not a constant feature. As well as attenuation of UV (wavelengths less than 309 nm) by true absorption (Moon 1940) blue light is selectively scattered according to Raleigh's Law, by molecules of nitrogen, oxygen and other gases and red light is selectively absorbed by water droplets. Under conditions of dense cloud, total irradiance may be reduced to one-tenth of the value on cloudless days (Fritz 1957; Gates 1962; Monteith 1973) (see Figure 4.1). Factors such as latitude, season and time of day affect the angle of incidence of the sun's rays, and their duration. They affect the "optical air mass" which at sea level is the ratio between the slant path length of the sun's rays through the atmosphere, and the zenith path length. This is numerically equal to the secant of the sun's zenith angle (Gates 1962; List 1951) (see Figure 4.2). The air mass is effectively an indication of the amount of air which must be traversed by the sun's rays, and this naturally has an effect on the attenuation due to the atmosphere at the point in question. Specifically, Coblentz & Stair (1944) found that the amounts of UV of wavelengths less than 313 nm reaching the ground in the tropics was greater than in temperate latitudes due to the smaller mean air mass at the former sites. At Ganzirri, at midday in June, the air mass is 1.04 and in December it is 2.2. The corresponding values for Durness are 1.2 and 6.5 (computed from solar altitude data of List 1951).

Solar radiation exists as two components, the "direct solar" component, emanating from the sun's disc, and the "diffuse sky" component consisting of

the blue skylight. An object situated in full sunlight is irradiated by the total of these components with on average about 80% due to the direct component. An object in full shade is irradiated by diffuse sky radiation alone, unless the shading object has modified the sun's spectrum by transmission as occurs beneath plant canopies. The relative proportions of sun and sky components change with the sun's elevation and with the time of year.

In addition to these factors, marine plants live below a water column whose changing optical characteristics may outweigh all others in ecological importance (Kain et al. 1975). Pure water absorbs all radiation strongly except for a "window" of high transmission between 200 nm and 700 nm, coinciding closely with PAR wavelengths (Morel 1974). Very "clean" ocean waters, e.g. Sargasso Sea or East Mediterranean, have spectral qualities approximating those of distilled water (see Figure 4.21). Sea salts alter the spectral properties negligibly, but the presence of any suspended solid matter completely alters the situation, causing (Raleigh) scattering of the shorter wavelengths (Strickland 1955). Thus, pure water acts as a monochromator for blue light (Tyler 1959; Jerlov 1971) but the presence of solid material tends to shift the maximum transmission towards the longer wavelengths. Jerlov (1951, 1970) after intensive studies on tropical, subtropical and temperate waters produced a classification scheme comprising three oceanic water types and nine coastal types, based on energy attentuation and spectral transmission characteristics. The maximum transmission of light by coastal waters is in the green region (e.g. coastal type III - 525 nm, Figure 4.20) which is the region of minimum absorption by chlorophyll, but is absorbed by carotenoid pigments such as fucoxanthin and the phycobilins which occur in quantitatively important amounts in the Phaeophyta and Rhodophyta respectively. This

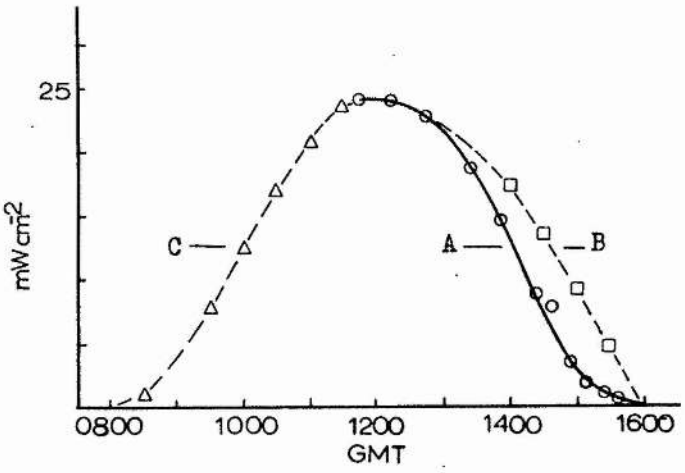
occurrence has been used as circumstantial evidence (e.g. Rabinowitch 1951) for the vindication of Englemann's (1883) theory of complementary chromatic adaptation, seeking to explain the occurrence of red and brown algae in greater quantities than green algae in the sublittoral region, because their pigment absorption spectra are matched to the spectrum of irradiance transmitted by the water. The question is a difficult one to resolve because of the difficulty in assessing the success of the various phyla. On a biomass scale, for instance, the massive Phaeophyta represented by the Fucales and Laminariales make nonsense of the argument that the Rhodophyta are in any way "dominant", although species of the red algae do indeed predominate at the extreme lower limit of the photic zone in British coastal waters (Norton 1968; Norton & Milburn 1972).

In the present chapter, results are presented of measurements of available surface light at the various surface sites, and of the attentuation of this light in British waters. Some preliminary measurements of the spectral composition of the irradiance available are also included. The results are discussed in relation to geographical and hydrographical conditions and to the photosynthesis and growth of macroalgae.

2. Surface Irradiance

(a) The diurnal light cycle

When measured under constant conditions, the variation of irradiance over the period of one daylength is sinusoidal. This is illustrated by measurements made on a clear afternoon in St Andrews in December, using the Lintronic integrator read half-hourly (Figure 4.3). Under conditions



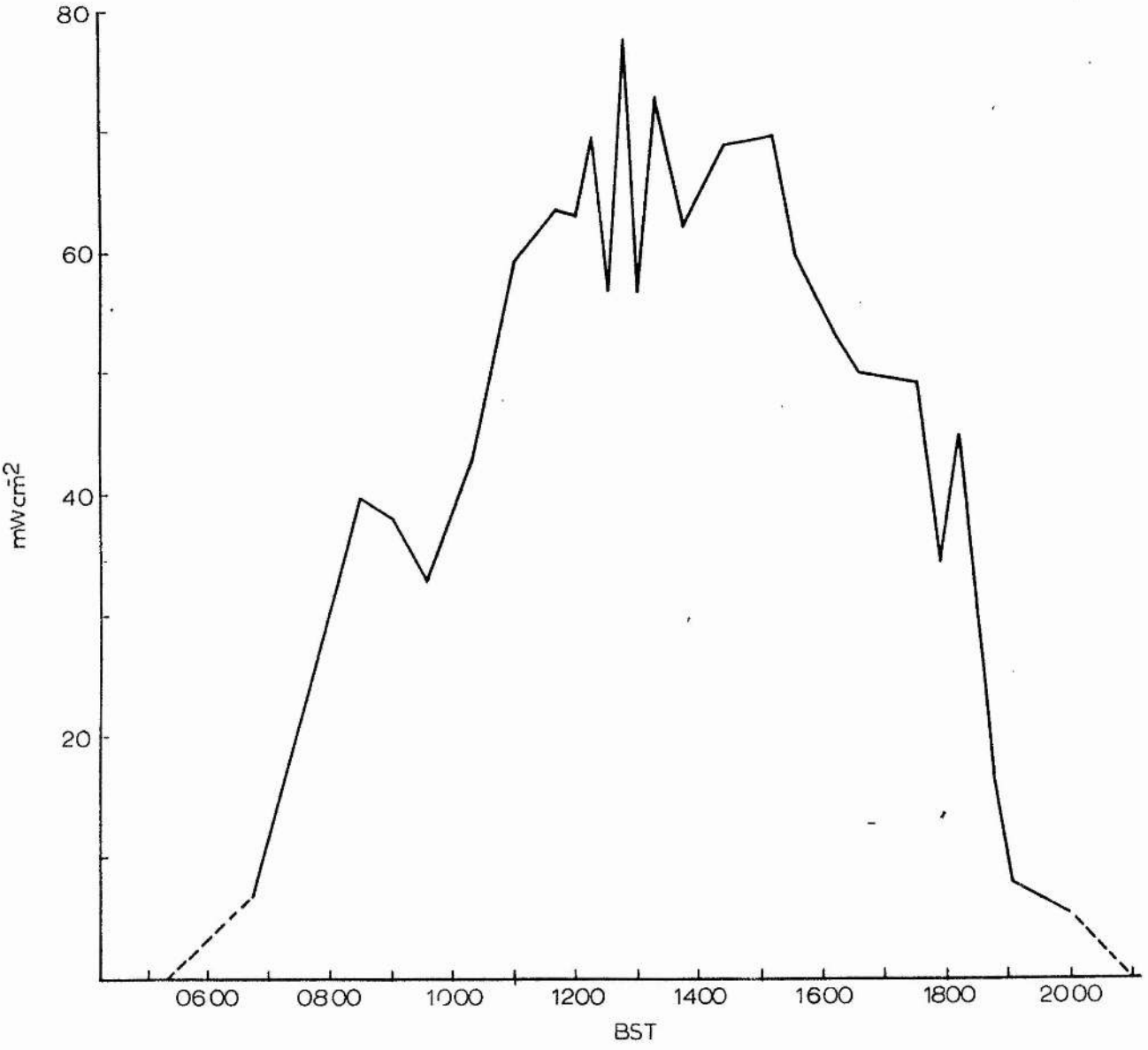


Figure 4.3. (upper). The diurnal light cycle; A, measured on a cloudless afternoon at St. Andrews in December; B, calculated from equation 4.1; C, calculated from equation 4.2.

Figure 4.4. (lower). Diurnal light cycle for a day of broken cloud, at Eilean Hoan, August.

of intermittent cloud and clear sky, a less continuous, "spiky" curve is produced as similar readings for a whole day at Durness (Eilean Hoan) in August showed (Figure 4.4). The noon maxima were 24.3 and 77.2 $mWcm^{-2}$ for St Andrews and Durness respectively.

Because of the regular nature of the diurnal insolation curve, diurnal curves can be constructed mathematically, for days of uniform irradiance, from a knowledge of daylength and either total irradiance integrated over the day in question, or the noon maximum irradiance. Monteith (1973) gives the following equation for the solar curve:

$$S_t = S_{tm} \sin (\pi t/N) \qquad (4.1)$$

Where,

S_{tm} = noon maximum irradiance ($J\ cm^{-2}\ h^{-1}$).

N = daylength (h)

S_t = irradiance ($J\ cm^{-2}\ h^{-1}$) at time t (h) after sunrise

$\pi t/N$ is in radians. Curve B in Figure 4.3 shows the shape of this curve derived from a noon maximum irradiance of 87.5 $J\ cm^{-2}\ h^{-1}$ and daylength 8 h as found in curva A. It is clear that this approximate relation is not a good fit to the empirical data.

Vollenweider (1965) characterised the "standard light day" clear and cloudless) by the equation:

$$S_t' = S_{tm} \tfrac{1}{2} \left(1 + \cos \frac{2\pi t'}{N}\right) \qquad (4.2)$$

Where,

t' = Time (h) measured positively or negatively with regard to

zero time taken at local midday.

For the same December situation, this yields curve C in Figure 4.3 which appears to be a good fit to the mirror image of the empirical curve A.

Noon irradiance values can be used, with a knowledge of the solar curve, to compute the total daily irradiance (T). Monteith (1973) gives the integral:

$$T = \frac{2}{\pi} N S_{tm} \qquad (4.3)$$

The symmetry of Vollenweider's relation yields the simpler relation:

$$T = \frac{1}{2} N S_{tm} \qquad (4.4)$$

which, in words, is equivalent to the product of the mean irradiance ($J\ cm^{-2}\ h^{-1}$) and the daylength (h). Table 4.1 shows values for total daily irradiance computed using these two methods compared with the empirically derived totals for the days in December and August.

Table 4.1 Computed and measured values of total daily irradiance

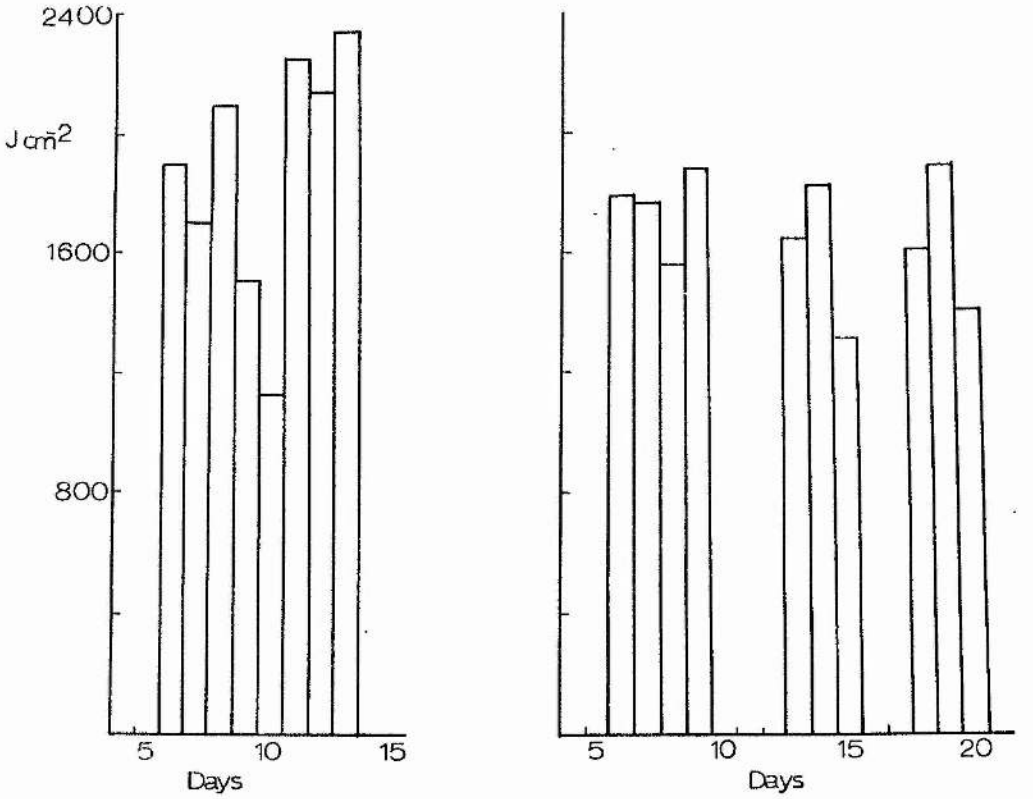
	Daylength	Noon irradiance	Total daily irradiance $J\ cm^{-2}\ d^{-1}$		
	h	$J\ cm^{-2}\ h^{-1}$	$\frac{2}{\pi} N S_{tm}$	$\frac{1}{2} N S_{tm}$	Measured
December, St Andrews	8.0	87.5	446	350	358
August, Durness	15.5	278.0	2743	2155	2099

Monteith's (1973) model (equation 4.3) can be seen to yield estimates of total daily irradiance which exceed the empirical values by as much as 30%. Vollenweider's (1965) model gives estimates within 2 to 3% of the measured values and appears thus to be most suited to the curves obtained by irradiance measurements in the present work.

Clearly, such analytical methods are approximations and can only be applied to days of near-uniform irradiance. Equations 4.2 and 4.4 have been used in the present study to derive irradiance estimates for certain days for which only limited data were available. In such calculations, approximate daylength values can be obtained for any latitude and date from the Smithsonian Meteorological tables (List 1951). Care must be taken to relate the time of the true solar noon to local time, e.g. noon occurs at 1300 h British Summer Time. Solar noon varies from 1200 h GMT over the year by a quantity known as the "equation of time" (see e.g. Whitaker's Almanack 1974), but since this does not exceed 15 min. in Britain, sunrise and sunset can be assumed to be equidistant from noon, for the purpose of calculations.

(b) Total daily irradiance - Britain

Measurements of integrated total daily irradiance or noon maximum irradiance were not made at Puffin Island or Dunstaffnage, but sample measurements made at Durness (Eilean Hoan) using Lintronic integrator serve as examples of the levels of irradiance to be expected in summer in Britain. Figure 4.6 shows total energy values for the month of August. The mean value was 1884 J $cm^{-2} d^{-1}$ (450 cal $cm^{-2} d^{-1}$) compared with the mean value for this month and location of 1152 J $cm^{-2} d^{-1}$ (275 cal $cm^{-2} d^{-1}$) quoted



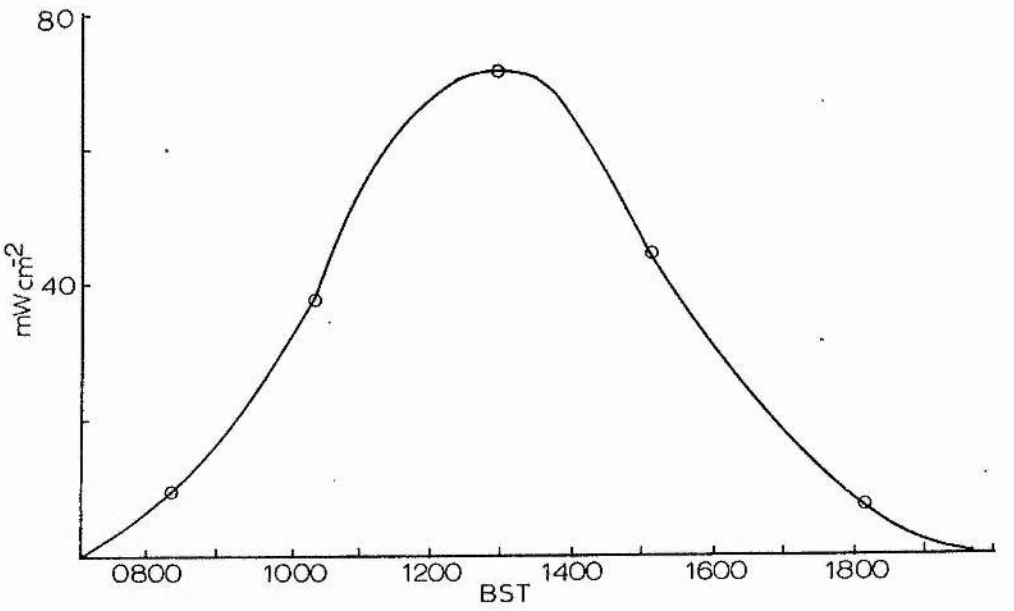


Figure 4.6. (upper, left). Total daily irradiance between 5th and 15th August, Eilean Hoan;

Figure 4.7. (upper, right). Total daily irradiance between 5th and 20th September, Ganzirri.

Figure 4.8. (lower). Diurnal light cycle for a cloudless day in September at Ganzirri.

by de Jong (1973), maximum irradiance was at noon on the 13th August when a value of 67.2 $mWcm^{-2}$ was recorded.

(c) Total daily irradiance - Ganzirri

Figure 4.7 presents measurements of irradiance made with the Lintronic integrator on days on which in situ experiments were conducted and for which complete daily readings are available. The irradiance was relatively invariable and the mean total energy was 1646 J $cm^{-2} d^{-1}$ (393 cal $cm^{-2} d^{-1}$) comparing closely with the mean figure of 1676 J $cm^{-2} d^{-1}$ (400 cal $cm^{-2} d^{-1}$) given by de Jong (1973) for this region of Sicily in September. Figure 4.8 shows an approximate daylight curve fitted to the data for the day of highest irradiance, September 9th, with a total of 1851 J $cm^{-2} d^{-1}$ and maximum irradiance (at noon) of 72 mW cm^{-2}. Similar curves could be drawn for any day recorded in Figure 4.8 and used for interpolation of values for calculations for in situ experiments.

It is worthy of note that although the maximum value for noon irradiance was recorded at Ganzirri, maximum total irradiance was found at Durness, due to the longer daylength at the higher latitude, 15.65 h compared with 12.40 h at Ganzirri.

3. Attenuation of irradiance in the sea at British coastal sites

(a) Data from "instantaneous" measurements using the selenium radiometer

Measurements were made as described in Chapter 2 at various British sites, within 2 hours of noon except on two occasions where the measurements

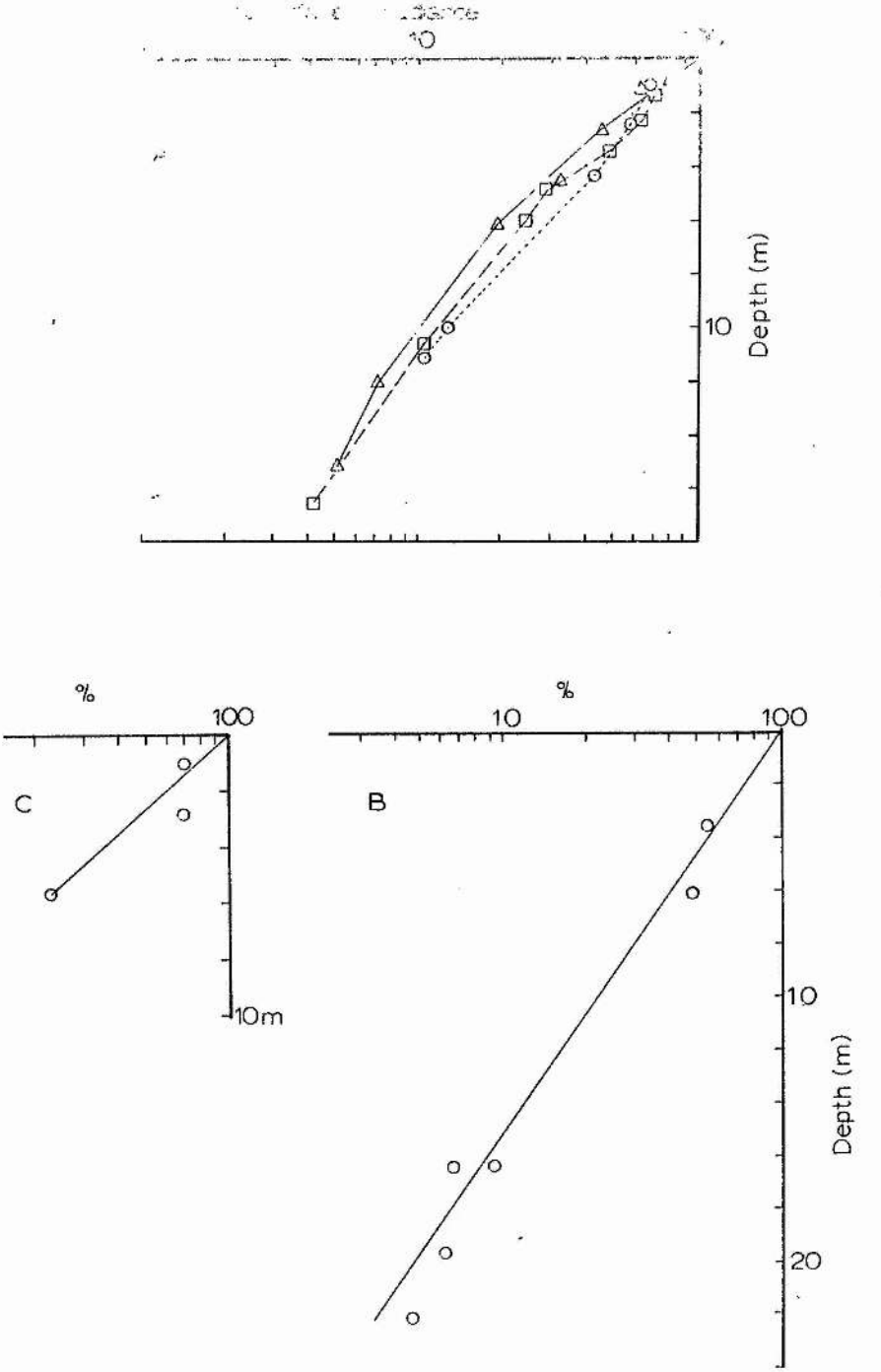


Figure 4.9. Attenuation of irradiance in the sea at Durness; A, on three days in August, Eilean Hoan site; B, on one day in March, Eilean Hoan site; C, on one day in June, Rispond, 1800h BST (semilogarithmic plots).

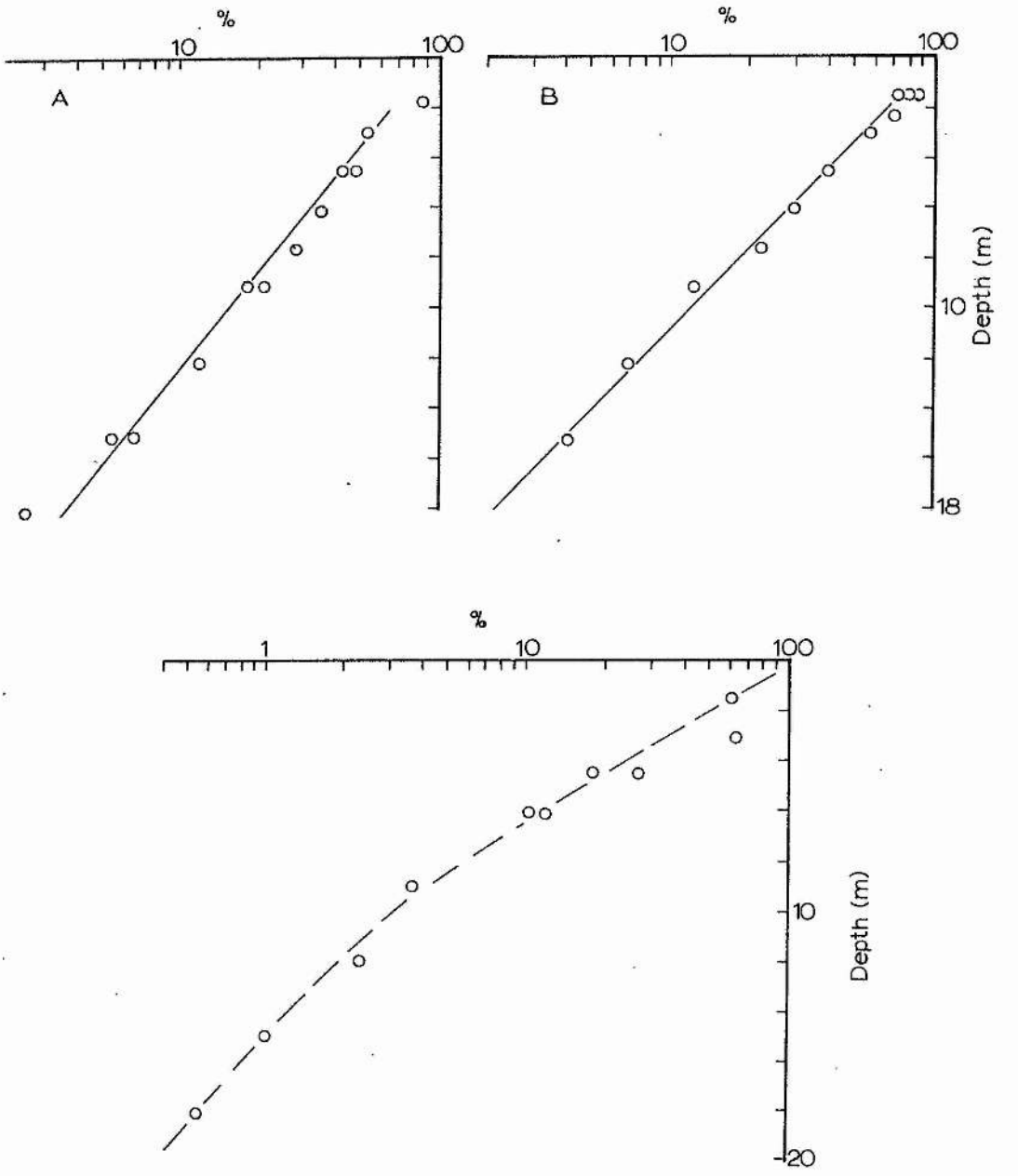


Figure 4.10. (upper). Attenuation of irradiance in the sea at Puffin Island within two hours of noon on two days in July (semilogarithmic plot).

Figure 4.11. (lower). Attenuation of irradiance in the sea at Puffin Island at 1800h BST on one day in July (semilogarithmic plot).

were made at 1800 h BST to show the effect of low altitude of the sun. The results were calculated as percentages of the measured subsurface irradiance or a computed value of it (subsurface = 80% of above-surface reading). The results are presented as semilogarithmic plots in Figures 4.9 - 11. In all cases the decrease in irradiance is shown to be close to logarithmic, but, at least in the detailed study at Durness (Eilean Hoan) shown in Figure 4.9, the lines curve constantly towards the ordinate, as shown in the classic studies of Jerlov (1951). The departure from perfect logarithmic relationship may be due to the decreasing component of oblique light as depth increases (Jerlov 1951; Strickland 1958). The relationships are strikingly similar for the summer months at Puffin Island and Durness (Eilean Hoan) with approximately 10% of subsurface light penetrating to 10 m. In March at Durness, however, the corresponding value was 20% of subsurface energy which, together with the subjective evidence of 24 m underwater visibility, may be taken to be due to the water's freedom from plankton or detritus at this time of year, under calm conditions. The transect carried out in the early evening in June (Figure 4.9C) was done under sunny, clear conditions, and shows a higher rate of attenuation than the other curves, only approximately 8% of subsurface irradiance being present at 10 m. Similarly the transect made at Puffin Island at 1800 h BST shows a markedly greater attenuation than the other two curves for this area, only about 4% of subsurface irradiance penetrating to 10 m. The relationship between underwater irradiance and low altitude of the sun, and the effects of local topography, are discussed later.

The attenuation curves shown in Figures 4.9 - 11 can be compared with those of Jerlov (1970) for various coastal water types where minimum

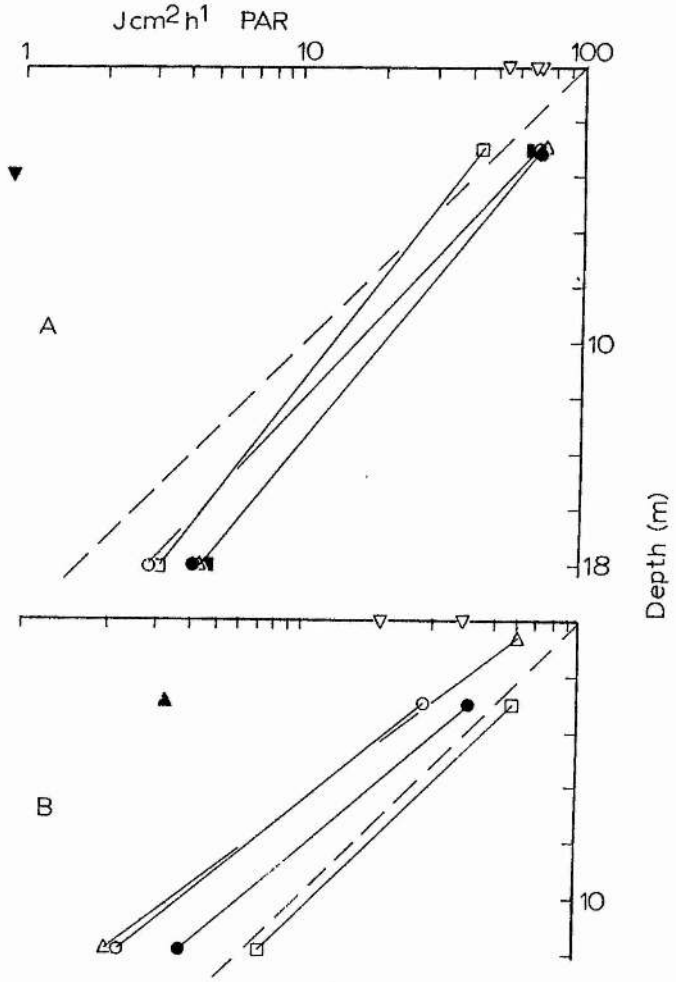


Figure 4.12. Attenuation of irradiance shown by integrated measurements made at two depths on different days; A, at Puffin Island, July (▼ signifies irradiance beneath L.hyperborea canopy); B, at Dunstaffnage, August (▲ signifies mean irradiance per daylight hour, calculated from irradiance integrated over a 27h period). (Semi-logarithmic plot).

attenuation was recorded in type 1, with 13% of subsurface irradiance between 400 and 700 nm being present at 10 m.

(b) Integrated measurements using selenium integrators

Integrated measurements were made over the time course of *in situ* experiments at Puffin Island and Dunstaffnage. Results were expressed as Joules $cm^{-2} h^{-1}$ PAR and are shown in Figures 4.12A and B on a semilogarithmic plot. For comparison, the approximate relationship found from the instantaneous measurements has been added as a dashed line. Firstly it should be noted that the reason for relatively low readings at 0 m is that surface experiments tended to be carried out when inclement weather conditions precluded diving operations and consequently irradiance was low due to overcast skies. Quantitatively, the *in situ* values indicate that over a period of about 4 h, during a month of high irradiance and under good weather conditions, approximately 4 J $cm^{-2} h^{-1}$ are available for photosynthesis at 18 m. This is equivalent to a mean irradiance of 1.11 mW cm^{-2} and is at a critical level with respect to limitation or saturation of algal photosynthesis by light, as will be shown later (Chapter 7). At Dunstaffnage, irradiance at 12 m was lower than at Puffin Island due to both higher attenuation and lower surface irradiance due to overcast conditions.

On one occasion at Dunstaffnage (Figure 4.12B), when irradiance at 3 m was integrated over a 27-hour period, the calculated hourly irradiance for a 14.38 h daylength (List 1951) was 3.23 J $cm^{-2} h^{-1}$ PAR (0.90 mW cm^{-2}) implying a noon maximum of twice this value. Because this value is so much lower than that obtained during short-term measurements at this depth, it seems likely that the effective daylength is substantially shorter here than above the surface, as suggested by Jerlov (1951).

Although no reference can be made to surface irradiance, an indication of the attenuation properties of the waters can be given by comparing the gradients of the lines joining parts of readings taken concurrently at the two depths at each site. When expressed numerically, such gradients are identical to the term called variously the vertical extinction coefficient (Strickland 1958), vertical attenuation coefficient (Westlake 1965) and diffuse attenuation coefficient (Hemmings 1966; Spence et al 1971). The latter term is favoured here since the light regime under consideration is of a diffuse nature. Although in the present case the coefficients could be derived directly from the curves themselves, coefficients are generally calculated from the basic data. The basis of the calculation is a statement of the laws of Bougier and Beer concerning the path of radiant energy through an absorbing medium and given by Westlake (1965) in the general form

$$I_m = I_o \ (\log \text{base})^{-EM} \qquad (4.5)$$

which can be rearranged to give

$$E = \log_{10}\left(\frac{I_0}{Im}\right) \cdot \frac{1}{m \log_{10} (\log \text{base})} \qquad (4.6)$$

Where E is the diffuse attenuation coefficient, I_0 the initial measured irradiance and I_m the attenuated radiation after passing through a depth m (usually expressed in metres) of water. A logarithm base of e (2.72) is frequently used (e.g. Spence et al.1971). If, however, a base of 10 is used, then the equation simplifies to

$$E_{10} = \log_{10}\left(\frac{I_0}{I_m}\right) \cdot \frac{1}{M} \qquad (4.7)$$

Using this equation, values for the diffuse attenuation coefficient (E_{10})

were calculated for the pairs of values appearing in Figures 4.12A and B, also for two of the transect curves, and a series of values of E_{10} were calculated from Jerlov's coastal water curves (Figure 4.15A). The values are shown in Table 4.2.

Table 4.2 Diffuse attenuation coefficients (E_{10}) for PAR, for coastal waters determined in the depth ranges 3m - 12m and 3m - 18m

		E_{10} (3m - 12m)	E_{10} (3m - 18m)
Coastal type	1 (Figure 4-15A)	0.0731	0.0701
	2 " "	0.1070	0.1010
	3 " "	0.1600	0.1560
Selenium integrator data			
Puffin Island			0.0845
Dunstaffnage		0.1110	
Transect data			
Puffin Island	(Figure 4.10A)	0.0780	0.0780
Durness (Eilean Hoan)	(Figure 4.9A)	0.0816	0.0763

The values of E_{10} for coastal types 1, 2 and 3 illustrate that lower values are associated with clearer waters. Considering the integrator data, the relatively high coefficient for Dunstaffnage indicates that this site is intermediate between coastal types 2 and 3, whereas the lower value for Puffin Island is close to coastal type 1. From the transect data and the values for the coastal types, it can be seen that although a perfect logarithmic relationship (as fitted to Puffin Island data, Figure 4.10A)

gives a constant E_{10} value, the "bending" curves for Durness (Figure 4.9A) and the coastal types (Figure 4.15A) necessarily show a decrease in E_{10} with depth. Due to this changing value of E_{10} at depths of less than 100 m, Strickland (1958) has underlined the undesirability of reporting average values for water masses. The use of the coefficient in the present case was principally to permit comparison between the Dunstaffnage water, for which no irradiance transect data were available, with the other waters investigated.

4. Reduction of irradiance by L. hyperborea canopy

(a) Puffin Island

During the course of an experiment in situ to measure the effect of the L. hyperborea canopy on photosynthesis of typical underflora algae, light measurements were made using the selenium integrators, situated simultaneously on the experimental platform above the canopy and beneath the canopy on the rock, at the bases of the L. hyperborea plants. The results were as follows:

Irradiance above canopy, depth 2.7 m = 43.6 J $cm^{-2}h^{-1}$

" below " " 4.1 m = 0.91 J $cm^{-2}h^{-1}$

In order to assess the reduction of light due to the canopy, the energy of light liable to be present at 4.1 m depth in open water must be computed:

Irradiance in open water, depth 4.1 m = 0.79* x Irradiance at 2.7 m

= 0.79 x 43.6 J $cm^{-2}h^{-1}$

= 34.4 J $cm^{-2}h^{-1}$

$$\text{Fraction penetrating canopy} = \frac{0.91}{34.4} \times 100 = 2.65\%$$

This reduction is seen in comparison to other absolute energy values in Figure 4.12A.

(b) Durness (Eilean Hoan)

Here instantaneous irradiance readings were used to calculate the reduction of light by the canopy at slightly greater depths. The results were:

Irradiance above canopy, depth 4.8 m = 5.37 mW cm^{-2}

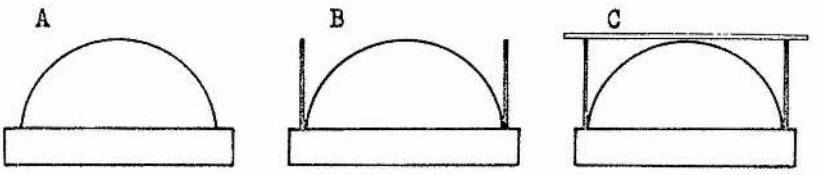
" below " , " 6.1 m = 0.56 mW cm^{-2}

Irradiance in open water, depth 6.1 m = 0.76* x Irradiance at 4.8 m
= 0.76 x 5.37 mW cm^{-2}
= 4.08 mW cm^{-2}

$$\text{Fraction penetrating canopy} = \frac{0.56}{4.08} \times 100 = 13.8\%$$

This result is also plotted on Figure 4.9A to allow comparison with the transect values. While based on only two observations, the results illustrate the effect of the canopy in greatly increasing the attenuation of irradiance reaching the underflora and give an indication of the range of this effect in different kelp stands due to depth and geographical variation. It should also be noted that although the mean irradiance below the canopy is thus reduced it can for brief intervals, due to movement of the kelp fronds, approach that ambient in open water at the same depth.

* These values are from Figures 4.9A and 4.10A respectively



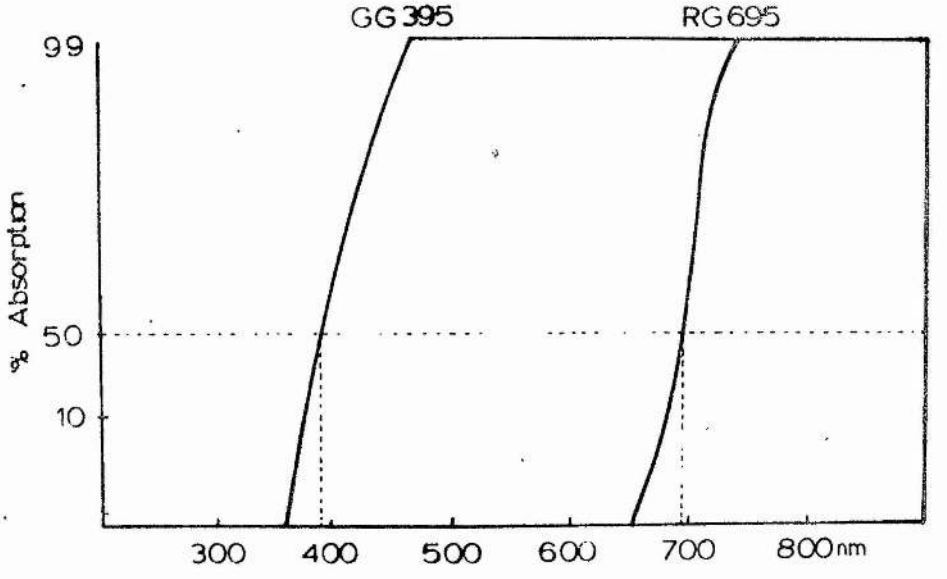


Figure 4.13. (upper). Modification of dome collector of Lintronic solarimeter for measurement of UV, PAR and IR radiation; A, normal collector;B,black card collar in position for total energy measurement; C, glass filter in position resting upon collar.

Figure 4.14. (lower). Spectral absorption characteristics of UV-absorbing (GG 395) and IR-transmitting (RG 695) optical filters.

5. Spectral energy distribution of solar irradiance

Using the Lintronic solarimeter (Chapter 2) and suitable filters, an attempt was made to measure the relative contributions to the total surface solar irradiance made by the ultraviolet, "visible" or PAR, and infrared spectral regions. The detector was modified by the addition of a black card collar, to receive flat glass filters (Figure 4.13). The filters used were a UV absorbing filter GG395 and an infrared transmitting filter RG695, made by Schott-Jens, Mainz. Their spectral properties are shown in Figure 4.14. Total irradiance was measured with no filters present. No measurement was made of reflection losses caused by the filters, but readings taken with the filters present were corrected for a total loss of 4% as suggested by the spectrophotometric measurements made with the incubation bottle glass (Figure 2.11). Although it must also be assumed that the thin (1 mm) glass dome fitted over the thermopile absorbs some 7% (from Figure 2.11) of incident radiation between 300 - 350 nm, no correction was made for this to the apparent UV values. Measurements were made on two days in August at Durness (Eilean Hoan). One day had clear sky with some high altitude cirrus clouds which periodically reduced irradiance, the other day was foggy with a hazy sun sporadically visible. On each day readings were taken in early afternoon in about six consecutive groups of three, i.e. without filter, with filter GG395, with filter RG695. Meter readings obtained with filters present were corrected by division by 0.96, the UV fraction was obtained by subtraction from the total reading for that group of three, and both UV and IR expressed as percentages of this total reading. Radiation from 388 - 695 nm was obtained by subtraction and the absolute energies computed from a total irradiance reading made with the solarimeter without the masking ring.

The results are presented in Table 4.2.

Table 4.2 Spectral energy distribution of solar irradiance at Durness (Eilean Hoan) in August

	Clear Sky		Foggy	
	%	mW cm^{-2}	%	mW cm^{-2}
UV (300 - 388 nm)	0.40 ± 1.26	.34	7.47 ± 2.63	.896
Visible (388 - 691 nm)	47.30 ± 1.34	40.20	43.70 ± 5.52	5.240
IR (691 + nm)	52.30 ± 0.87	44.50	48.80 ± 2.31	5.860
Total	100	85	100	12

The IR fractions were relatively consistent around 50% of total irradiance, with a slightly smaller proportion on the foggy day as is to be expected due to the high attenuation of long wavelength radiation by water droplets (Gates 1962). The UV results however were extremely variable, several of the values obtained on the sunny day having negative values. These results were thus unacceptable and those for UV on the foggy day were in considerable doubt. There are two main reasons for such variability of the UV measurements.

(a) the proportion of irradiance below 395 nm is so small that any slight variation in the total irradiance whilst taking a reading with filter GG395 in place could account for more than the absolute value for UV irradiance. This fault could clearly be overcome by taking simultaneous readings with two solarimeters.

(b) there may be unknown errors due to changes in the geometry of the collector with the filters present. An underestimate of the reflection losses due to the filters would result in an underestimate of IR and overestimate of UV and vice versa. It is possible that the lowered value of IR and

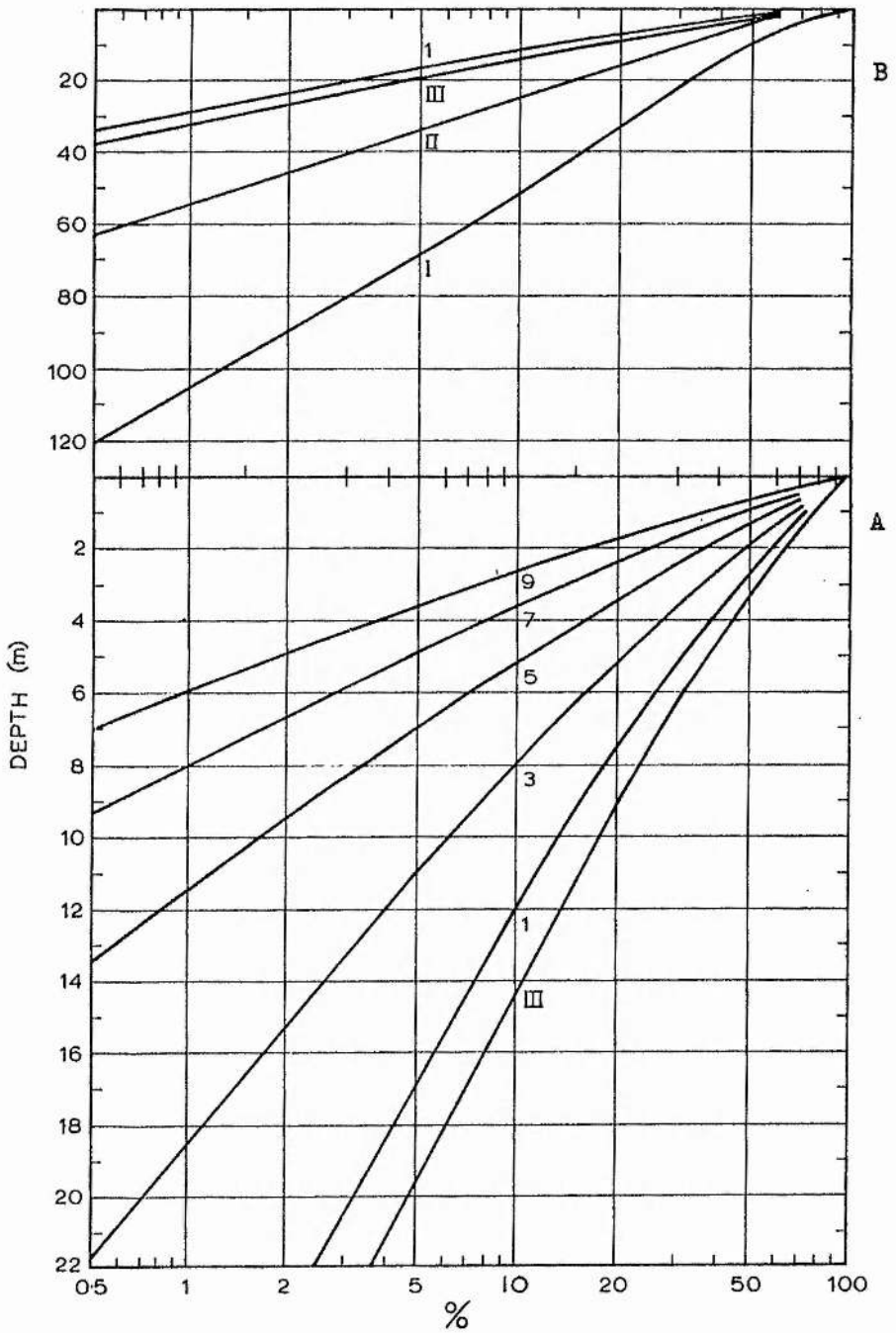


Figure 4.15. Attenuation of surface irradiance by seawater of classified types A, coastal types 1, 3, 5, 7 & 9; B, oceanic types I, II & III (semilogarithmic plots; from Jerlov 1970).

raised value of UV on the foggy day are due to underestimation of the excessive reflection losses which may be incurred due to the low angle of incidence of much of the fog-scattered irradiance. It is concluded that though the method is probably satisfactory for determining IR total irradiance (or PAR total) ratios the small amount of energy present below 400 nm render the method impracticable for measurement of UV.

6. Discussion

Because attenuation of irradiance by the water column is the most important single factor affecting the light climate of sublittoral algae it will be discussed here first. The attenuation data for British waters presented above compare well with the coastal water types proposed in the classic studies of Jerlov (1951) for depths greater than 5 m. In depths shallower than 5 m however, the data of this author indicate a very high attenuation and in Jerlov's clearest coastal type (type 1) only 27% of the subsurface irradiance penetrates to 2 m compared with 50 - 60% in the present work. Recently Jerlov has published an up-dated version of his water type classification (Jerlov 1970) which show the original curves moved closer to the ordinate axis, removing the initial high attenuation effect and producing attenuation values very similar to the present work (Figure 4.15A). It is interesting to note that this close comparability occurs in spite of the different methods used to collect the data, since Jerlov's measurements were made in open water using a photometer suspended from a ship. In detailed studies made at the Isle of Man, Kain (1965, 1971)

and Kain et al.(1975) have laid stress on the transience of hourly and daily irradiance measurements and emphasised the importance of integrated longer term (e.g. monthly) readings. Kain (1965) found an absolute minimum attenuation of 10% surface irradiance (400 - 700 nm) penetrating to 10 m, which is closely comparable with the Durness water (Figure 4.9A). Water clarity was occasionally found to be as good in December as in June during calm weather (Kain 1971), but winter storms resulted in higher mean attenuation by the water column. Of more importance to the fixed benthic plants, however, is the integrated amount of light energy reaching a sublittoral habitat as a function of state of tide and time of day, as well as water clarity. Clearly the integrated irradiance falling upon a surface in the sea will, over a period of years, correspond to the attenuation caused by the mean depth and mean irradiance at the sea's surface. Within any month however, time and tide may conspire to produce high or low extreme values. Thus at sites where low water spring tide habitually occurs in early afternoon, the high irradiances produced can modify the species distribution of the sublittoral flora (Biebl 1959). Kain et al.(1975) obtained a July daily mean of 19% surface light penetrating to a photocell mounted 5 m below lowest astronomical tide, representing a mean depth of 7.5 m. This value is similar to those recorded above for Puffin Island and Durness at 7.5 m but the daily range at the Isle of Man of 5 to 30% is stated as being due in part to the varying tidal heights. In the winter months, Kain et al (1975) frequently recorded values as low as 1 to 5% at 7.5 m mean depth, corresponding to Jerlov's coastal types 5 to 7, but the mean monthly level of about 10% for winter over four years corresponds to type 3. It is probable that this pattern is similar for all unpolluted sites round Britain as confirmed by the results for summer months in the

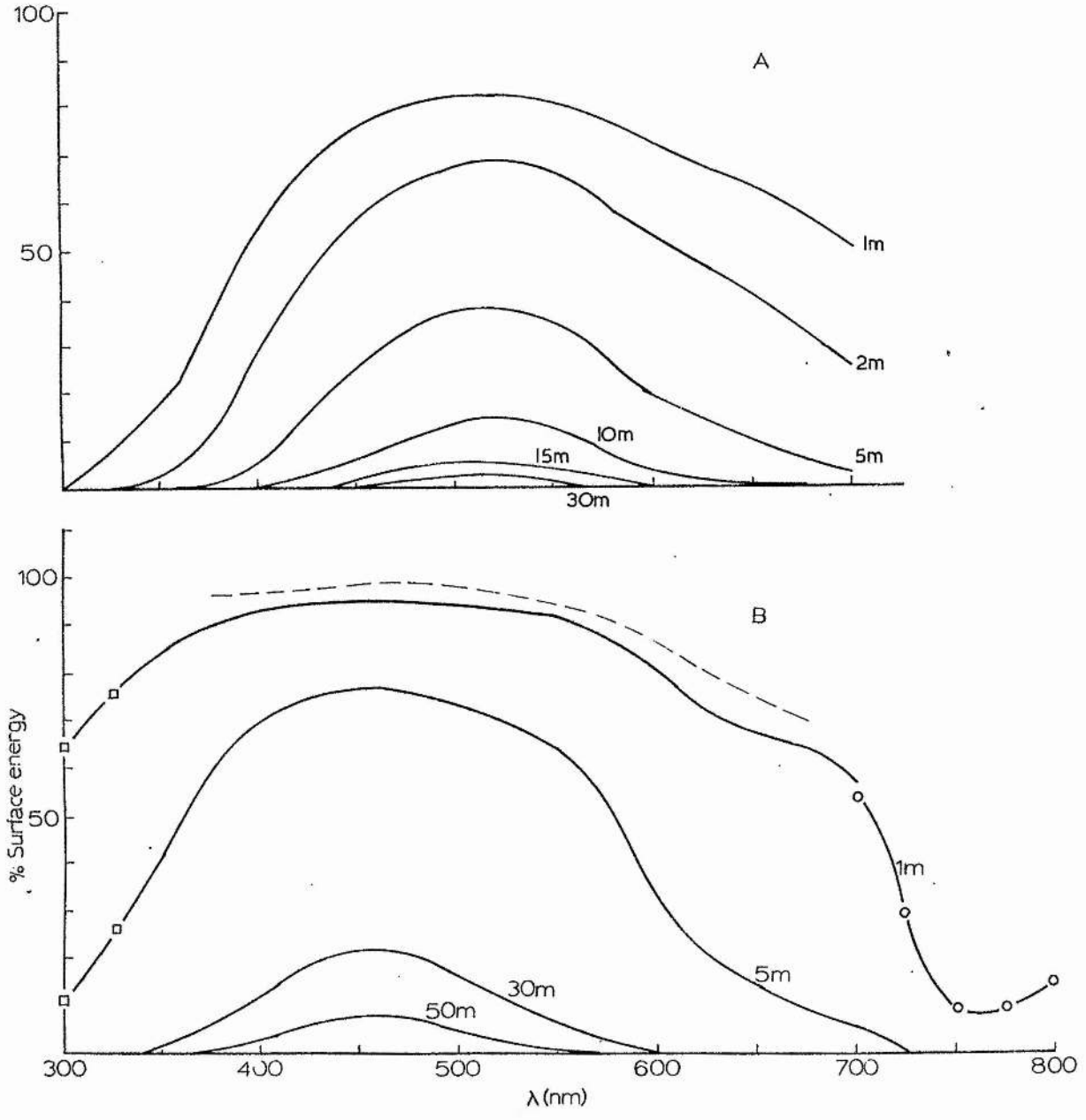


Figure 4.16. Spectral energy distribution of irradiance calculated for various depths in; A, coastal water type 3; B, oceanic water type II. Main portions of curves from Jerlov (1970); □ , from Jerlov (1951); ○ , from Strickland (1959); broken curve represents spectral irradiance beneath 1m of pure water (Jerlov 1970).

present study, but the influence of large inputs of water from rivers with their accompanying silt, and, frequently urban effluent, can dramatically alter coastal waters' attenuation locally. Foster & Morris (1974) found that in the Liverpool Bay area "organically dirty" water was positively correlated with low salinity due to the influence of the River Mersey. It is certain that the presence of humic material from the large watershed of Loch Etive (Droop et al 1973) is responsible for the higher attenuation of light measured at Dunstaffnage compared with either Puffin Island or Durness, where nearby rivers are small.

The attenuation characteristics of the water at Ganzirri were not measured in the present study but Drew (1972) and Giaccone (1972) measured 0.6% and 1.0% respectively of surface light at 50 m suggesting an attenuation similar to that of Jerlov's type II or III oceanic water (Jerlov 1970; Figure 4.15B). Mediterranean waters have frequently been given oceanic status (Jerlov 1951; Hemmings 1965; Lythgoe 1965) and calculations in this thesis concerning attenuation at Ganzirri have been based on the oceanic type II curve.

The change in spectral quality of light with increasing depth in the sea is a well known phenomenon (Levring 1947, 1965; Jerlov 1951, 1970; Smith & Jones 1971) and has long been accorded importance in the utilisation of underwater light energy by benthic algae (e.g. Fritsch 1945). Figure 4.16A and B shows the spectral distribution of irradiance at different depths in type 3 coastal and type II oceanic waters with wavelengths of maximum transmission at 525 nm and 475 nm respectively. Although the spectral characteristics of ocean waters are largely dictated by those of the water itself, the characteristics of coastal waters are further modified by the effects of particulate inorganic and organic matter and dissolved organic

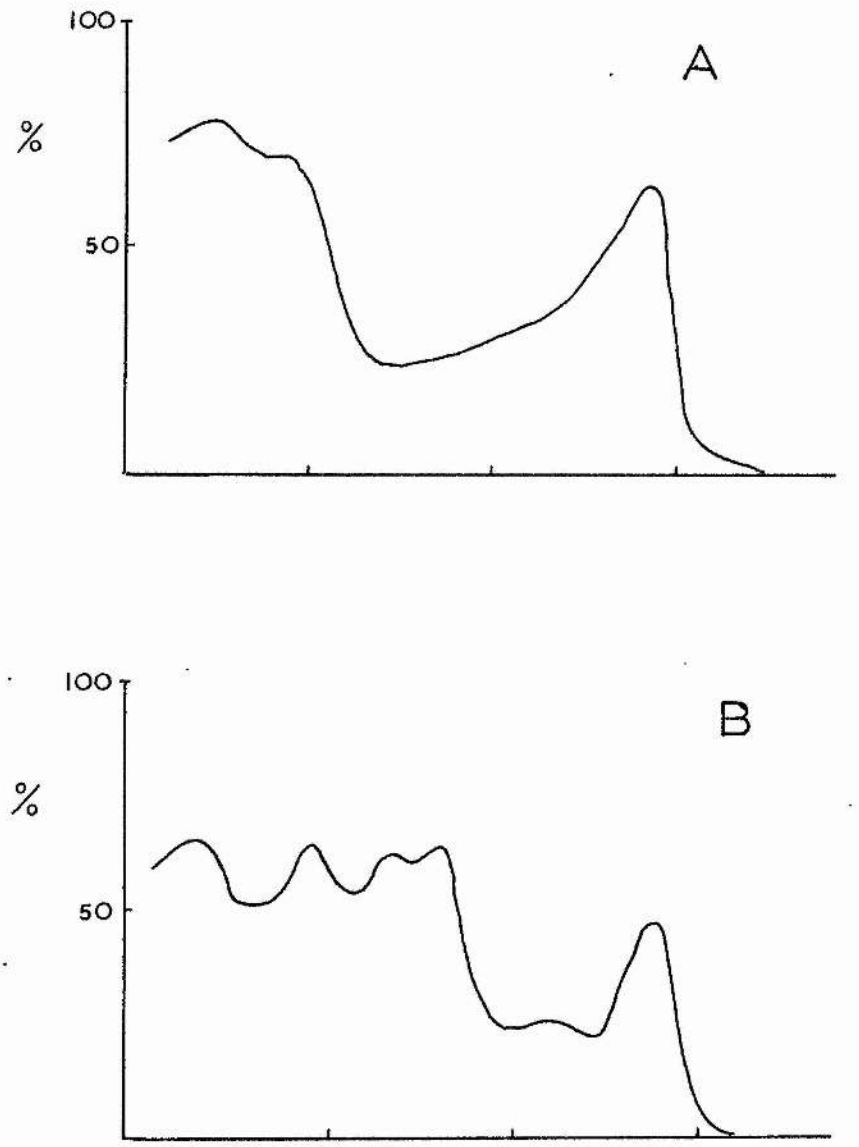


Figure 4.17. In vivo absorption curves for thalli of: A, Ulva taeniata; B, Delesseria decipiens (from Lax & Blinks 1950).

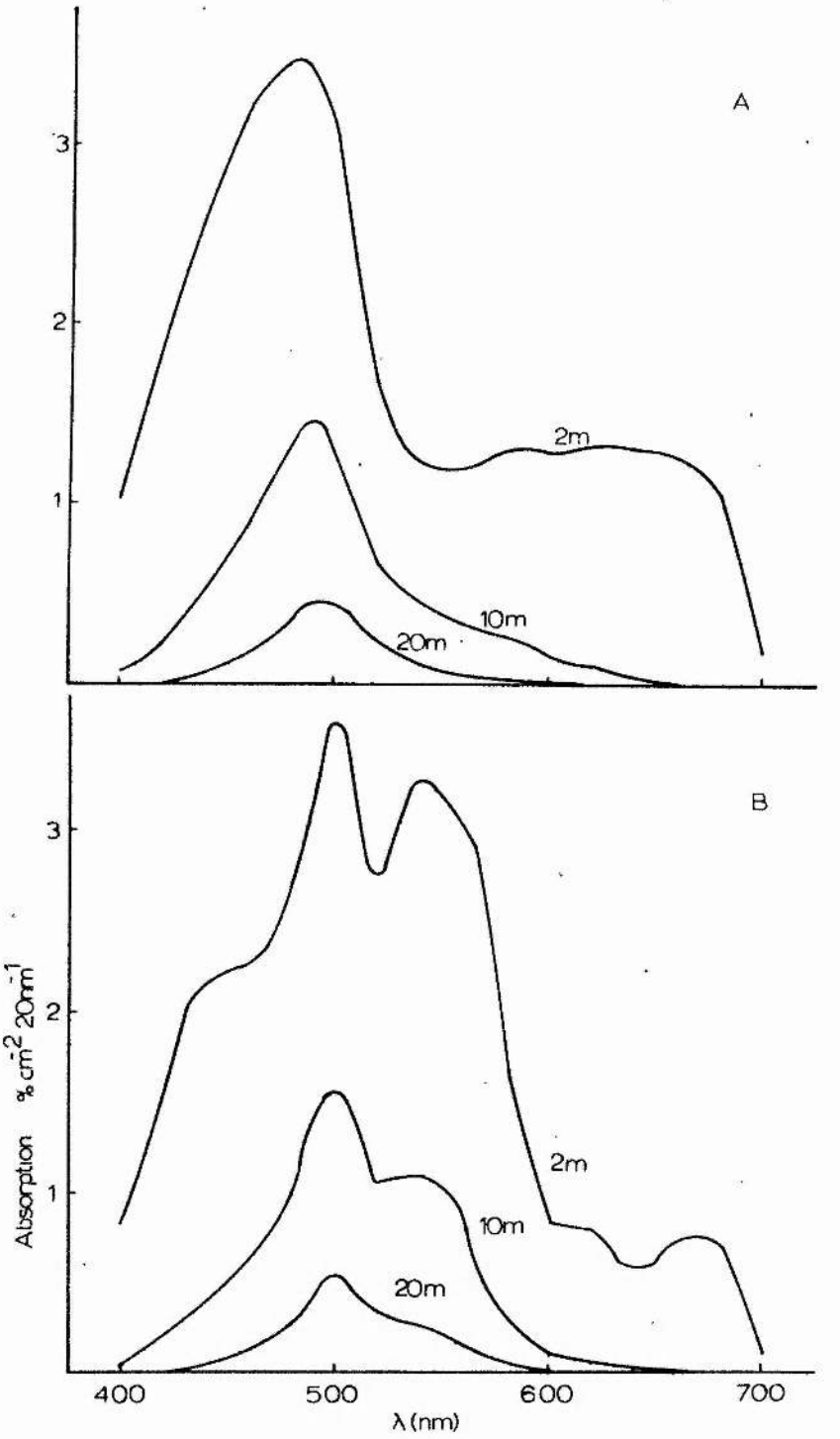


Figure 4.18. *In vivo* absorption curves for algal thalli at various depths in seawater of coastal type 1, extrapolated from data in Figure 4.16 &17; A, *Ulva taeniata*; B, *Delesseria decipiens*

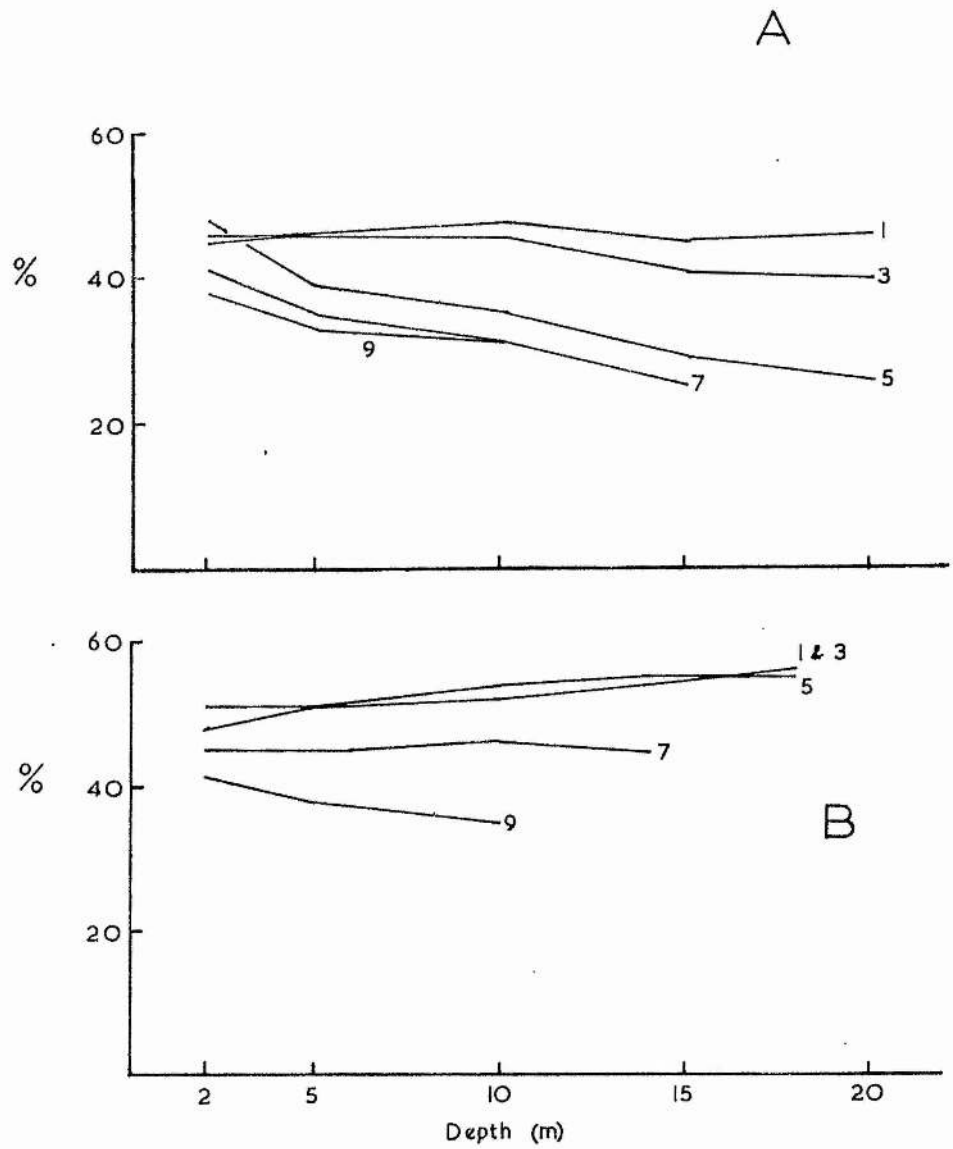


Figure 4.19. % of ambient irradiance (total PAR) absorbed by algal thalli *in vivo*, at different depths in different types of coastal water; A, *Ulva taeniata*; B, *Delesseria decipiens*.

matter (Jerlov 1951, 1971; Strickland 1958; Kullenberg 1974). Inorganic particles (mostly silica) scatter shorter wavelengths. Suspended particulate organic matter scatters and absorbs shorter wavelengths while transmitting or non-selectively absorbing longer ones. It is composed mainly of dead phytoplankton and terrestial material, but Yentsch (1963) has stressed that the absorption of blue light by living phytoplankton makes a significant contribution to the attenuation at this end of the spectrum. Dissolved organic matter, "yellow substance" or "gelbstoff" has distinct components of marine and terrestrial origin and is composed of breakdown products of cellulose, amino acids and lignin; it absorbs strongly below 500 nm (see review by Kalle 1966). Popular chromatic adaption theory (Rabinowitch & Govindjee 1969) suggests that red algae have an advantage over the green at greater depths due to their phycobilin pigments which absorb energy in the green part of the spectrum, i.e. light which is transmitted by coastal waters. Figure 4.17A and B show in vivo absorption curves of the thalli of the green alga Ulva and the red alga Delesseria re-drawn from Haxo & Blinks (1950). Figures 4.18A and B show curves extrapolated from the above information illustrating the effective energy absorption spectra of these two species at different depths in coastal type 1, showing how the influence of chlorophyll in Ulva is progressively reduced as depth increases. The total absorptions of the thalli, however, are surprisingly similar quantitatively (Figure 4.19A and B) especially in clearer water, Delesseria absorbing 56% of available energy at 10 m in coastal type 1 while Ulva absorbs 48%, only 8% less. The absorption of green light by Ulva and other members of the Chlorophyta, is due to the presence of caroteniods as well as chlorophylls. Similar calculations (Crossett & Larkum 1965) for oceanic type II water showed that absorption by Ulva was relatively greater than shown here for coastal water and was equal to that of a red alga (Porphyra)

due to the absorption of the transmitted blue light by chlorophyll. All these calculations suggest that no great advantage is conferred on red algae *a priori* by the possession of phycobilins, but further work would require to be based on absorption spectra of plants collected from deep sites, as all plants used by Haxo & Blinks were shallow-grown specimens.

On the basis of results from *in situ* photosynthesis experiments, Gail (1922) suggested that a significant reduction of light transmitted by the water-air interface of the sea occurs during rough surface conditions. Strickland (1958) states that apart from any reflective surface losses, there is an unexpectedly high attenuation in the first few metres in rough weather possibly due to scatterance and reflection by the layer of bubbles always present in a disturbed ocean surface. Reflection losses themselves are relatively unaffected by rough weather at angles of incidence greater than 45°, only about 2% of incident irradiance being reflected under all surface conditions (Jerlov 1951; Austin 1974). In calm weather however, angle of incidence can have a great effect, and at a solar altitude of 10° (attained e.g. at noon, December, Durness) reflection loss of direct solar radiation would rise from 2.1% to 27%, although any surface movement would result in much smaller reflection losses (Jerlov 1970). Also, Spence (1976) emphasised that when solar altitude is low, the diffuse sky radiation component of daylight is correspondingly greater and this may result in a fairly constant proportion of daylight being transmitted through the interface to the underwater environment. The results of attenuation transects carried out in early evening (Figures 4.9C and 4.11) suggested that once through the surface, irradiance is attenuated more strongly at low altitude of the sun. This effect was also found by Jerlov (1951) who ascribed it to an increase in the mean path length underwater of the sun's rays. However,

Smith & Jones (1971) found identical attenuation of irradiance in coastal waters measured at noon and a half-hour after sunrise and before sunset. As suggested earlier, it was felt that the topography of adjacent land might have been affecting the evening light readings at Durness and Puffin Island. In this connection, Aas (1967), working in a Norwegian Fjord in January, found that because the sun did not rise above the fjord sides during the course of the day, only blue skylight was available at the water surface. In addition to reducing available light markedly, this had the effect of shifting the underwater spectrum towards the blue wavelengths.

Concerning the collection of irradiance by the algae, little is known of the in situ, in vivo properties of algae, but most workers suggest that absorption is generally less per unit area than is found in most land plants (Levring 1947; Haxo & Blinks 1950). This may not signify a functional inefficiency however, since one reason may be that the algae, with water-filled thalli, transmit more light than the higher plants with their equally wasteful light-scattering air spaces (Rabinowitch 1951; Packer & Deamer 1968). The mechanism of re-orientation of the chloroplasts of algae may be important in determining the absorption properties of individual thalli depending on irradiance availability. Senn (1916) recorded parastrophe (Chloroplasts lining walls parallel to light rays) and diastrophe (chloroplasts lining walls perpendicular to light rays) in Peysonnellia squamaria under different conditions of irradiance whilst after two hours in sunlight, chloroplasts of the red alga Platoma cyclocolpa were parastrophic but in "diffuse" light they were diastrophic. Spence (1976) noted similar responses in Potamogeton crispus with shallow (o.25 m) leaves exhibiting parastrophe and deep (2.5 m) leaves showing diastrophe and transmitting 5% less light (400 - 700 nm). Such less well understood phenomena as the light induced changes in transmission of thalli of Ulva and Porphyra caused by

alterations in chloroplast structure (Packer & Deamer 1968) may also have ecological significance.

The geometry of algae as light collectors has also not been described. Monteith (1973) has suggested that terrestrial plants (e.g. trees) may approximate to spheroids or cylinders in their light collection properties. It is probable that a similar model would suffice for erect algal forms, i.e. Laminaria hyperborea, especially when their movements due to swell are integrated in three dimensions. Flat thallus forms occurring in areas of little water movement (e.g. Ulva, L.saccharina, Dilsea) may approximate more to 'cosine response" collectors. When considering the irradiance available to the "spheroid" model, values obtained using cosine collectors must be increased by a certain factor to include all available irradiance irrespective of direction. Thus, in very turbid coastal water, for depth down to 20 m, vertical irradiance values may have to be increased by 50%, although 30% is probably a reasonable average (Jerlov 1951; Strickland 1958).

Self shading plays an important role in the light regime of any dense plant community and L.hyperborea undoubtedly plays a large part in the sublittoral zonation of the smaller benthic algae in temperate waters. Measurements of the shading effect of the kelp canopy were first made by Kitching (1941) who found only 1% penetration of irradiance at shallow depths (1 - 4 m) but up to 15% at 11 m. The figure for shallow kelp forest is close to the 2.7% found at Puffin Island, but values in the literature are generally much higher. Thus Smith (1967) reported that at 3 m (below extreme low water springs), 33% of available irradiance penetrated the canopy and this proportion steadily decreased to 5% at 9 m (although the density of L.hyperborea was seen to decrease also). Jupp (1972) recorded 8% penetration at 3 m at Fife Ness but 33% at 6 m at Durness which is

Also why does LAI fall if PAR doesn't?

closer to the 28% reported as a mean value for forests at the Isle of Man (Kain 1965). It seems likely, therefore, that the light reduction by the canopy decreases with depth (apart from Smith's results) due to the reduction in the number of canopy-forming individuals of L.hyperborea per m^2 and concomitant reduction of lamina area index (LA I). On the west coast of Scotland, Robertson (1970 and other unpublished data) found a maximum of LAI of 12.5 m^2 lamina m^{-2} substrate at 3 m depth whereas at 10 m a maximum of 5.7 m^2m^{-2} was found. Geographical differences probably preclude any generalisation about the effect of the kelp canopy unless plant density is known. Kain et al.(1976) have suggested that the reduction of LAI with depth intereacts with the attenuation of irradiance with depth to produce a sub-canopy light regime which is relatively constant with depth. Working in the Adriatic Sea, Zavodnik (1971) measured the reduction of light by the canopies of several small-stature plant communities (less than 0.5 m high), by uplifting quadrat samples and reconstructing them on shore on a nylon net above a photometer. Cystoseira communities, a common deominant of the upper sublittoral in the whole Mediterranean (see Chapter 5) transmitted from 1.1 - 5.2% of incident irradiance, a figure closely comparable with those obtained for L.hyperoborea in temperate waters.

Although mean or integrated measurements of irradiance beneath algal canopies are important in terms of the photosynthetic requirements of the underflora, values for maximal irradiance may be necessary when assessing such plant responses as photoinhibition by intense light. The short term fluctuation of irradiance below laminarian canopies has been remarked upon by various authors (Kitching 1941; Smith 1967; Neushel 1971; Aleem 1973) and is due to the constant movement of the algal fronds producing "holes"

in the canopy leading to a "sunfleck" effect. Smith (1967) found a short-term variation of 5-fold in shallow (around 2 m) L.hyperborea forests, the variation decreasing with increased depth. Neushel (1971) also found a 5-fold variation but this was beneath a Macrocystis canopy at 10 m depth. In addition to the influence of canopy movement, plants living close to the water's surface can be exposed to enormous fluctuations of light intensity on sunny days due to the focussing effect of surface ripples (Cox 1974). Thus, for short periods, shade plants of the underflora may be exposed to irradiances outside their long-term tolerance range.

It seems reasonable to expect that the light climate beneath the kelp canopy is changed spectrally, as well as quantitatively, as was first suggested by Kitching (1941). No data are available for marine situations, but in the terrestrial analogy, light below forest canopies has been found to be rich in green and red wavelengths, corresponding to lgiht transmitted by the leaves (Whitmore 1966; Vezina & Boulter 1966).

In addition to a knowledge of the attenuation properties of the body of water under consideration, note must be taken of the absolute amounts and the quality of radiation reaching the sea's surface. In the tropics, irradiance as high as 3770 J $cm^{-2} d^{-1}$ (total radiation, 280 - 3000 nm) are recorded in midsummer (in Chile and Australia - Newbould 1963, de Jong 1973) equivalent to a noon irradiance of 144 mW cm^{-2}. On a cloudless midsummer's day in Britain the noon irradiance could reach 90 mW cm^{-2} (Monteith 1973) and the value of 77 mW cm^{-2} quoted for Durness which may be up to 15% too low due to the presence of cirrus clouds (Gates 1962) is close to this. These figures are extreme maxima and their importance lies not so much in their contribution to photosynthetic fixation, but to possible

radiation damage. Table 4.2 above showed that from 47.7 to 51.2% of the total energy measured at the earth's surface is of wavelengths less than 690 nm. In a review of the literature, Szeicz (1966) found values of the ratio PAR : total radiation ranging from 40% (Gaastra 1958) to 65% (measurements made in Estonia). No measurements were available for Britain. Strickland (1958) stated that 53% of total radiation lay between 350 and 700 nm. The classic data of Moon (1940) showed 45% to lie between 400 and 700 nm. The values in Table 4.2 are within the range encountered by Szeicz (1966). Since there exists no absolute value for this ratio and in view of the changes in its value with differing climatic conditions, a nominal value of 50% for radiation between 400 and 700 nm, as recommended by Monteith (1973) has been adopted for calculations involving PAR in this thesis.

With respect to the infrared component of solar radiation, the absorption spectrum of chlorophyll has a "window" between 700 and 3000 nm, which may help to prevent overheating of algae (Rabinowitch 1951). Water on the other hand is extremely opaque to infrared, which may result in overheating of algae indirectly by rise in temperature of the bathing water, e.g. in rock pools, or directly by local heating of the thalli as suggested by Doty (1971). Such effects would only be of importance to algae exposed at low tide or submerged in shallow water. It may be noted here that the red (660 nm) : far - red (730 nm) ratio is greatly altered underwater, being always greater than the value of 1.3 for surface sunlight (Spence et al. 1971). This may be of importance if phytochrome is influential in algal morphogenesis (Dring 1974) and the great attenuation of far-red light may enhance the significance of the minor absorption bands of phytochrome around 450 nm (Dring 1971).

The values of irradiance below 388 nm shown in Table 4.2 are variable and in one case higher than those suggested in the literature. The standard solar curves proposed by Moon (1940) indicate that for an air mass of 1 (i.e. the maximum possible values) the irradiance due to wavelengths below 400 nm is 4.01 mW cm^{-2} constituting 4.3% of the total energy under clear conditions. Standard texts (e.g. Wyszecki & Stiles 1967 and Monteith 1973) quote figures of 3% of total energy. Vezina & Boulter (1966) found that the irradiance between 325 - 400 nm constituted 10% of that between 325 - 749 nm. From Figure 2 it can be calculated that the irradiance from 350 nm (approximate cut-off of Lintronic glass dome) to 388 nm (cut-off of GG 388) is in the region of 2% of the total irradiance under clear conditions, compared with the measured mean values of 0.4 and 7.47% in Table 4.2.

The ecological significance of irradiance below 400 nm has rarely been investigated, although Johnston & Levring (1946) first showed that littoral algae such as Polysiphonia and Enteromorpha could carry out gross photosynthesis at 366 nm. This spectral region, although containing significant absorption bands of photosynthetic pigments has probably been neglected in studies of photosynthetic assimilation because of the low energies present in natural sunlight, as shown above, and also the difficulty of producing UV from convenient laboratory light sources. Experimental work on the effect of UV radiation frequently concentrates on the effects of irradiance produced by germicidal lamps which produce monochromatic light of wavelength 254 nm, not of ecological significance since energy below 280 nm does not reach earth's surface (Robinson 1966; Green 1966). Jerlov (1951) emphasised the importance of the UV "B" radiation (280 - 315 nm) which, though present in small amounts, has quanta of high energy

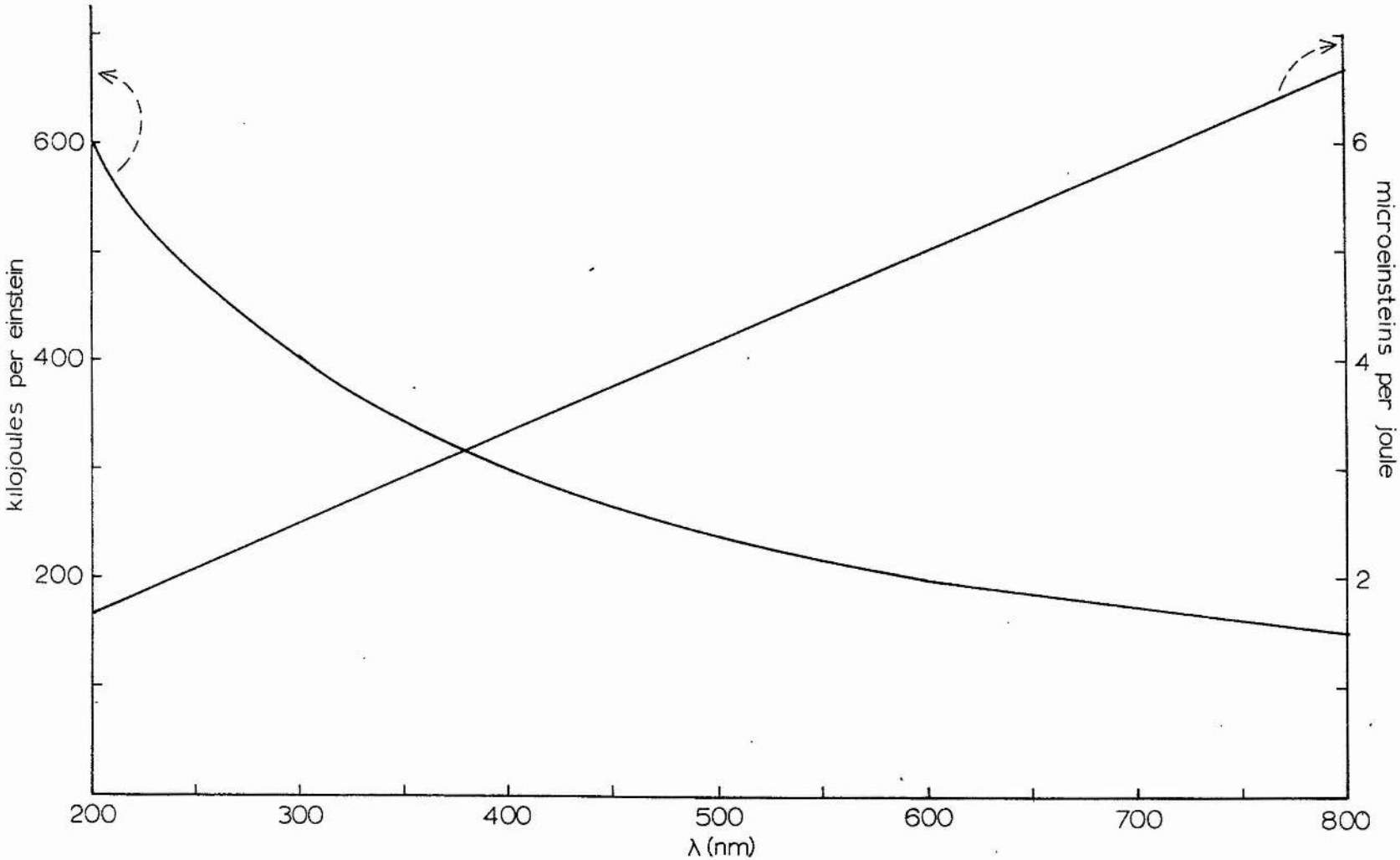


Figure 4.20. Relationship between energy and quanta in the region of the PAR spectrum.

and produces important biological effects at least in animals, e.g., the production of vitamin D and sunburn (peak sensitivity at 295 nm). He concluded that UV radiation effects on life forms could occur down to depths of 20 m in oceanic type waters but not in coastal types due to the strong absorption of UV by the "gelbstoff" (Figure 4.16A, B). The possible role of radiation below 400 nm in photoinhibition of photosynthesis is discussed in Chapter 7.

A note concerning quanta

The above discussion and results deal with irradiance and light availablity in terms of energy (J) or power (W) as measured by radiometers calibrated to respond equally (almost) to equal amounts of energy regardless of wavelength in the PAR region of the spectrum. Photosynthesis is a quantum reaction, however, and plants respond more in relation to numbers of quanta than total energy or power. Because of this, radiometers have been constructed which respond equally to equal numbers of quanta regardless of wavelength of PAR (e.g. Jerlov & Nygård 1969). Several recent papers have dealt with the spectral distribution of quanta underwater both in marine (Jerlov 1970; Halldal 1974; Steemann-Nielsen 1974, 1975) and freshwater (Spence 1975) habitats.

With increasing wavelength, the energy content of individual quanta decreases exponentially, and, conversely, the number of quanta per unit of energy increases linearly. Using the conversion factor from Šesták et al. (1971, p.417),

$$1 \text{ einstein} = \frac{12}{\lambda \times 10^{-7}} \text{ joules} \qquad (\lambda \text{ is wavelength in nanometres})$$

the relationships between energy and quanta have been calculated, and are shown in Figure 4.20.

The spectrum of irradiance above the surface of the sea has an approximately equal energy distribution between 400 and 700 nm (Rabinowitch

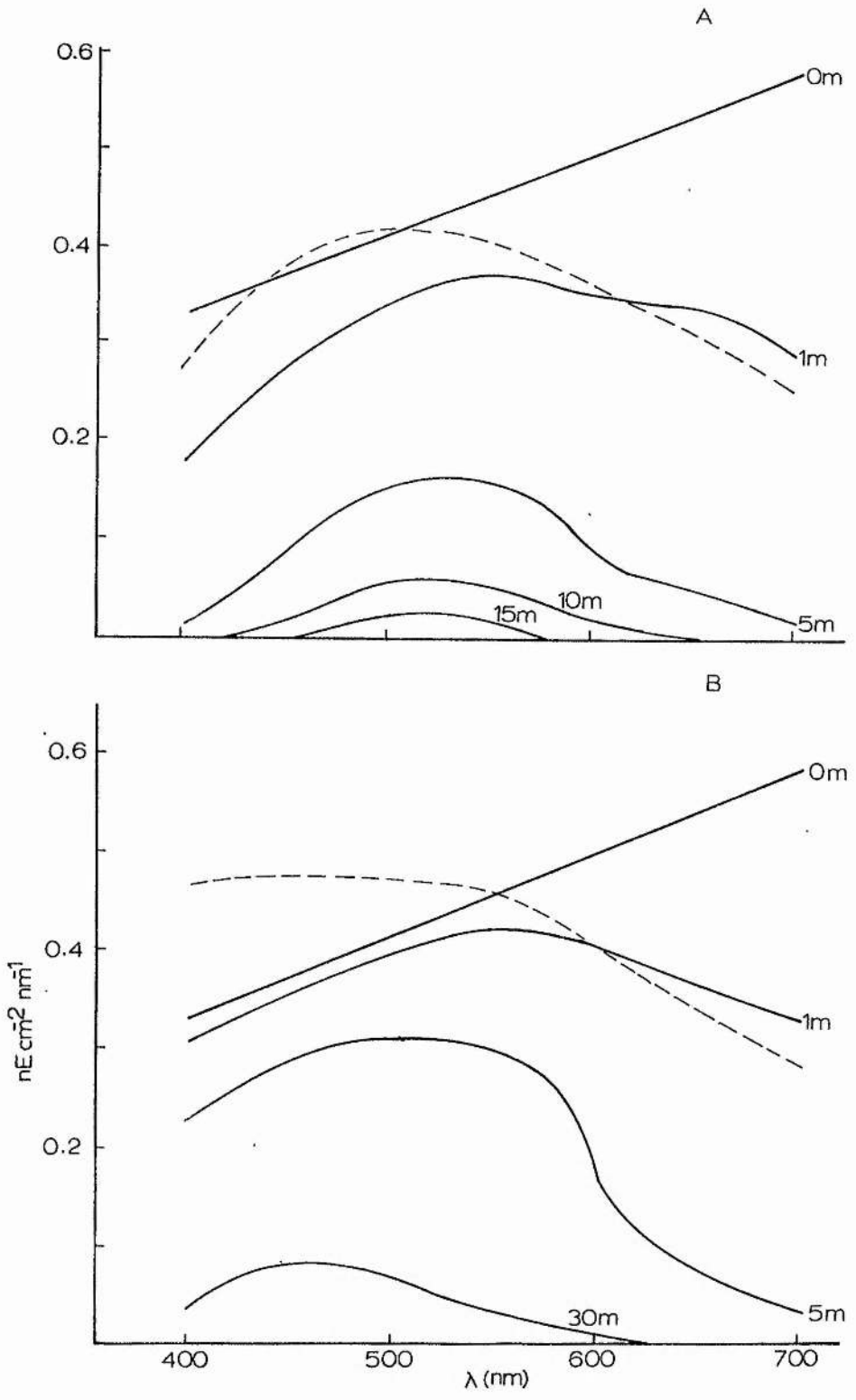


Figure 4.21. Spectral quantum distributions at various depths for an equal-energy spectrum at 0m; A, coastal water type 3; B, oceanic water type II. Broken curves show spectral energy distribution at 1m.

1951, p.732). On this assumption, the attenuation data of Jerlov (1970) yielded the spectral energy distributions at different depths in oceanic type II and coastal type 3 shown in Figure 4.16A and B with transmission maxima at 475 nm and 525 nm respectively. Because there are more quanta per unit energy near the red end of the spectrum, an "equal energy" spectrum is numerically richest in red quanta, and this is so in the case of the daylight spectrum at the sea's surface (Halldal 1974). Figure 4.21 A and B shows the spectral quantum distributions calculated for waters of coastal type 3 and oceanic type II. (For comparison, the spectral energy distributions at a depth of 1 m are also shown.) The quantum maximum shifts rapidly from 700 nm above the surface, to 500 nm in oceanic II and 550 nm in coastal 3 at 1 m depth, progressively shifting to the respective transmission maxima as depth increases. The figures show that, although the surface irradiance has a greater proportion of red light in quantum terms, the lateral compression of the spectrum with increased depth means that the disparity between the two modes of expression becomes less significant, as pointed out by Steemann-Nielsen (1974), In addition, the wide variation in the surface spectrum with time of day, weather and location (Halldal 1974), and differences in absorption and action spectra of the algae studied, where these parameters have not been measured, generally reduce the need for converting energy measurements into quantum equivalents in the present work.

CHAPTER 5

Ecology of sites and species

CONTENTS

1. Introduction

The aim of this thesis has been to measure macroalgal photosynthesis *in situ* and in the laboratory, and to correlate the findings with observations of algal distribution and with current theories of algal ecology. The present chapter is a largely subjective description of the communities from which the experimental material was taken, together with some quantitative data included to provide a background to the physiological experiments described in Chapters 6,7 and 8. It is not intended to be an exhaustive study of all the species present, but deals mainly with the experimental species and also certain notable dominant species. The positions of the sites are described, together with information regarding physical factors other than light (Chapter 4), such as water movement, temperature and substrate. Some preliminary measurements of biomass at Ganzirri and Durness are described.

Preliminary results are also reported concerning the effect of depth on specific lamina area (SLA = area of lamina per unit mass of lamina), phycoerythrin content and ash and energy content of certain species. Specific leaf area which is the terrestrial equivalent of SLA has long been noted as an adaptable characteristic of plants to sun and shade environments (Gabrielsen 1948; Björkman & Holmgren 1963; Coombe 1966) and its correlation with depth of growth of freshwater macrophytes has been studied by Spence & Chrystal (1970b) and Spence et al. (1973). Observations on the environmental modification of marine macroalgal thallus

structure, including SLA have been made by Norton (1969), Larkum (1971), and Svendsen & Kain(1971.) SLA has sometimes been expressed as its converse, "unit frond weight" signifying weight-per-unit-area (Larkum 1971), but SLA is the more accepted term, correlating closely with light collecting ability. Basically, an increase in SLA implies that the plant is "spreading its weight thinly over a large area" (Coombe 1966) and this is regarded as an adaptation to shade environments where light collection is a major limiting factor in photosynthesis.

Change in pigment content as a response to sun and shade environments has been noted in terrestrial plants (Gabrielsen 1948; Bjorkman & Holmgren 1963) and in algae (Brody & Emerson 1959) and is to some extent implicit in the chromatic adaptation theory in that red algae may increase their content of phycoerythrin when growing at deeper sites in response to the predominantly green irradiance ambient there (Rabinowitch 1945). Due to the difficulty of quantitative extraction of phycobilin pigments, most studies of phycoerythrin have been only qualitative, but quantitative work has been reported by Calabrese & Felicini (1970, 1973), Calabrese (1972) and Moon & Dawes (1976).

A short section on the ash and energy content of Sphaerococcus in relation to depth of growth has been included, facilitated by the very wide depth range (5 - 60m) of this species at Ganzirri.

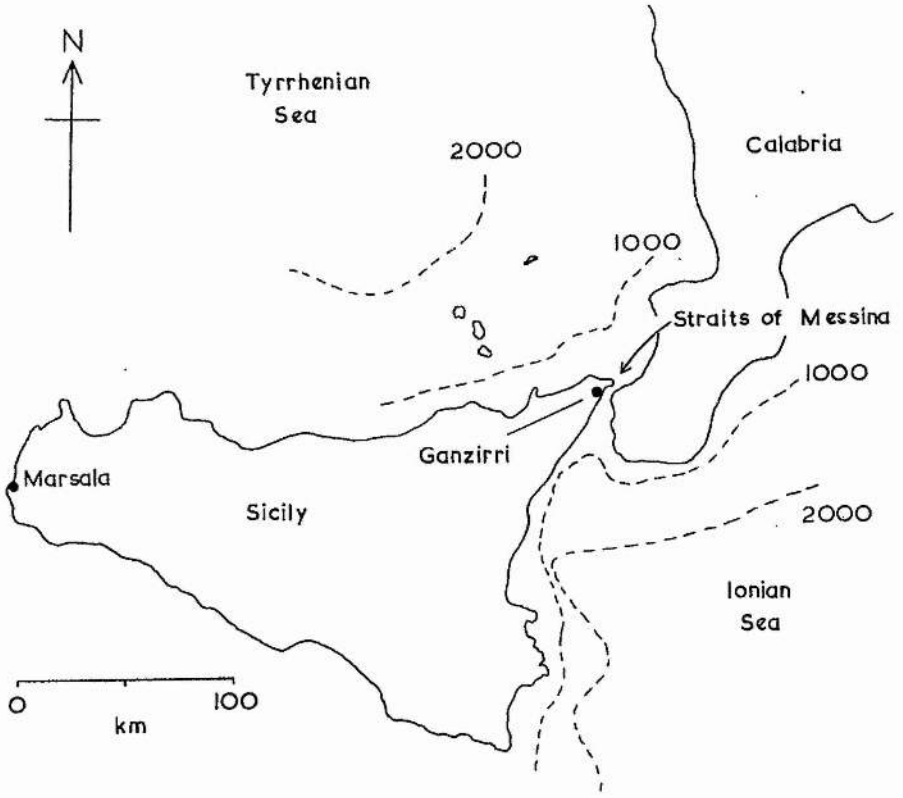


Figure 5.1. Location of Mediterranean experimental sites. Map shows 1000m and 2000m contours of sea floor north and south of Straits of Messina.

2. Description of sites

A. Mediterranean sites

a. Ganzirri - hydrology

The study area is situated off the fishing village of Ganzirri ($38^{o}15'N$, $15^{o}37'E$) 9km north of Messina on the Sicilian side of the Straits of Messina (Figures 1.2, 5.1). The area is subject to certain hydrographic peculiarities due to the opposing influences of the two adjacent major sub-units of the Mediterranean Sea, namely the Tyrrhenian Sea to the north and the Ionian Sea to the south. Due to a difference of about six hours in the time of high tide in these two seas, i.e. at opposite ends of the straits, substantial currents (maximum velocity $300cm\,s^{-1}$ = 6 knots) are generated in the 3km length of the straits due to water moving through to equalise the height difference, which reaches a maximum of 0.3m at spring tides. The currents have their greatest force where the straits are narrowest and shallowest which is between Ganzirri and Punta Pezzo (Admiralty 1965). At each turn of the tide (i.e. approximately every six hours) there is a brief period of slack water lasting from 20 minutes to 1 hour, known locally as "La stanca". Only during this period, could diving operations be safely carried out, and so *in situ* experiments lasted for the duration of one tidal flow.

Because of differences in the topography of the sea floor north and south of the straits (see Figure 5.1), the water entering from the Ionian Sea is of deeper origin than that from the Tyrrhenian Sea and is consequently colder and more saline. (There is a constant northward-setting current of about $10cm\,s^{-1}$ at depths below 30m, which is salinity-generated). The results of forty-six temperature determinations taken on separate days throughout the month of September are shown in Figure 5.2

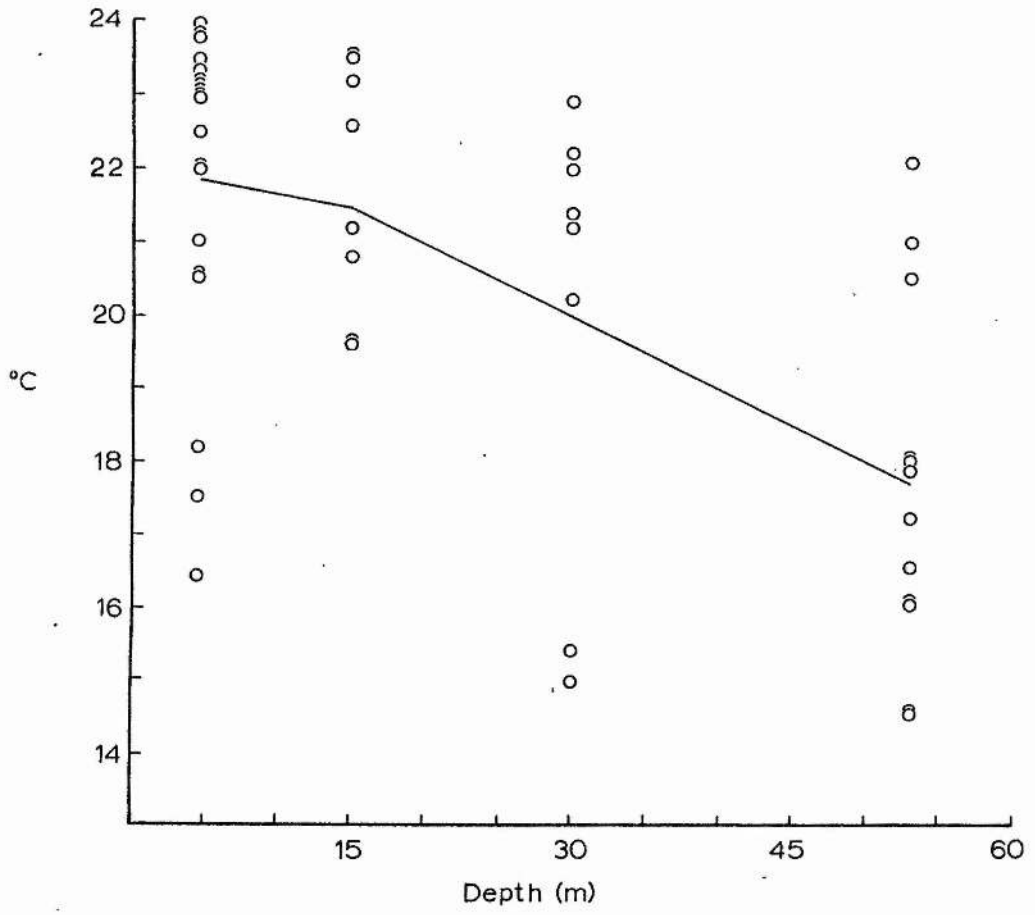


Figure 5.2. Temperature-depth profile at Ganzirri in September; determinations made at beginning and end of incubation periods.

in relation to depth. At each depth there was a tendency for values to be grouped at the upper and lower ends of the temperature range, rather than in the centre, as in a normal distribution. Although the range altered little with depth, the mean temperature and upper and lower extremes did decrease progressively with depth. Giaccone (1972) recognised that the temperatures of 14 - 17°C recorded in the straits were due to the deep water of the Ionian Sea, and this is probably the case in Figure 5.2, with the higher temperatures deriving from Tyrrhenian Sea water. In April, temperatures at all depths were very similar ranging from 14.1-14.8°C, presumably due to a reduction in temperature of the surface water of the Tyrrhenian Sea. In September, temperatures were measured at the beginning and end of each experiment and, usually one was low and one high; knowledge of the direction of current flow during any incubation period allowed the temperature which had been ambient throughout the experiment to be decided.

b. Description of the flora

The Mediterranean Sea belongs to the warm temperate biogeographical zone as classified by Hedgepeth (1957). A major difference between Mediterranean marine flora and that of Britain is the absence of laminarians in the lower littoral and sublittloral zones in the former waters. Due to the unique hydrography at Ganzirri, however, the sublittoral zone supports a flora different from and more productive than most other Mediterranean sites. The flora of the Straits of Messina has been described by Giaccone (1972), and includes the large laminarian species Sacchorhiza polyschides (Lightf.) Batt. at Villa san Giovanni, and Laminaria ochroleuca Pyl. at Ganzirri.

A schematic depth profile of the sublittoral zone at Ganzirri is shown in Figure 5.3. The substrate consists of sedimentary rock strata

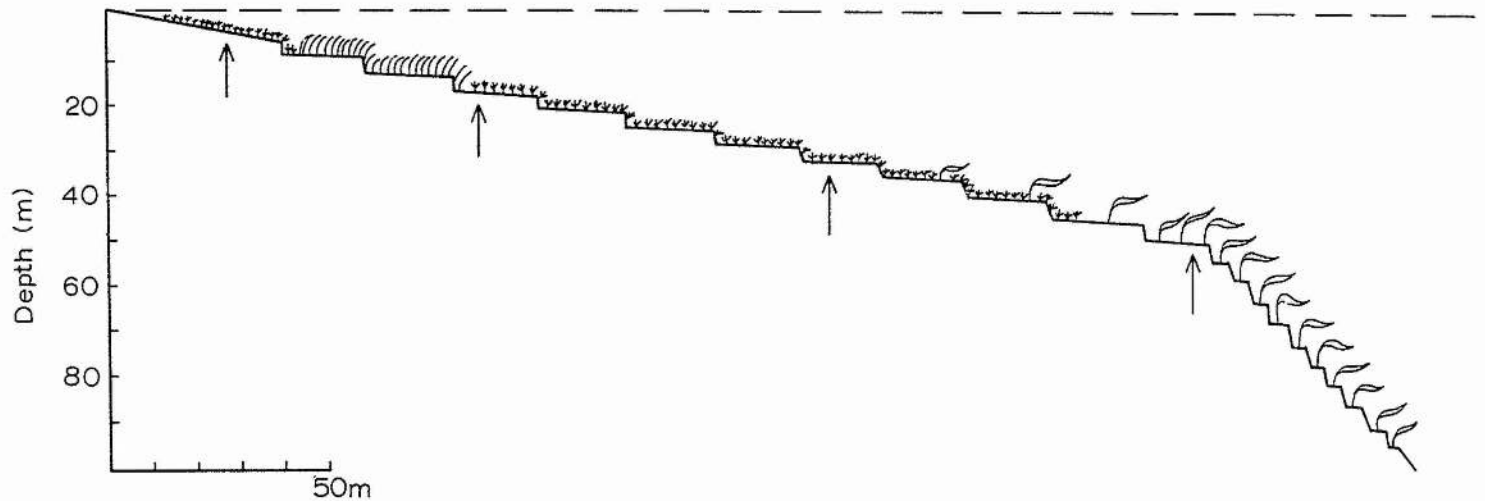


Figure 5.3. Profile of sea floor at Ganzirri showing dominant vegetation types; ⌇⌇⌇, small turf-forming algae; ////////, Posidonia oceanica; ~, Laminaria ochroleuca. Arrows mark experimental sites.

presenting a series of terraces gently sloping to a depth of 50 - 55m whereafter the sea floor descends more steeply, beyond the limit of compressed air diving, to 100m. The tidal range is approximately 0.3m and from this point to about 3m depth the substrate was sandy with patches of rock colonised principally by a thriving community of Ulva lactuca L. At 5m, a Cystoseira sp. community was well developed, with Ulva lactuca L. and Laurencia obtusa (Huds.) Lamour frequent. Gracilaria verrucosa (Huds.) Papenf. was found shaded by tufts of Cystoseira and depauperate but apparently healthy specimens of Sphaerococcus coronopofolius (Good et Wood) C.Ag. were found on two occasions. Dense beds of the marine angiosperm Posidonia oceanica (L.) Del. from approximately 8m to 12m precluded colonisation by algae except for small epiphytic forms. From the lower limit of the Posidonia beds to approximately 50m a dense cover of red and green algae was present dominated by Cladophora sp., Jania sp., Phyllophora sp. and Pterocladia capillacea (Gmel.) Born et Thur., but including Vidalia volubilis (L.) J. Ag. and Cystoseira. Juvenile Laminaria ochroleuca appeared at 33m but adult specimens were not encountered above 40m where their density was very sparse. Ulva and Sphaerococcus were conspicuous throughout the profile but were not dominant. The L.ochroleuca reached a maximum density (approximately $0.5m^{-2}$, Drew 1974a) at the break of slope region at 50-55m depth. Concurrently with the increase in density of this species, a decrease in cover by the smaller non-encrusting algal species (i.e. Jania, Cladophora) was noted, and the rock was increasingly encrusted with a Pseudolithophyllum expansum (Phil.) Lemoine community taking the form of calcareous overlapping plates growing prostrate on the rock substratum interspersed with sand patches and sparse individuals of Peyssonelia.

Sp., Sphaerococcus, Ulva and L.ochroleuca juveniles. Also present were individuals of the non-calcified red species Fauchea repens (C.Ag.) Mont. and the brown species L.ochroleuca (juveniles) and Phyllaria reniformis (Lamour) Rostafinsky. There were also frequent speciments of the shade alga Desmarestia dresnayi Lamour. ex Leman, this being the first record of this rare species in the Mediterranean Sea (Drew & Robertson 1974b).

In April, Ulva was absent from the deep sites (53-60m) but present at 4.5m although at a much reduced density. Experimental material of Sphaerococcus, Pseudolithophyllum and Peyssonelia was available in this month in similar abundance to September. A number of very small ($\sim 3cm^2$) specimens of Porphyra umbilicalis (L.) J.Ag. was found in April only, growing at a depth of approximately 1m.

In situ experiments were conducted at the sites marked at 4.5, 15, 30 and 53m (60m in April) in Figure 5.3. The sites at 15, 30 and 53m were marked by buoys during experiments to allow relocation, but that at 4.5m could be easily located by swimming from the shore.

c. Biomass data

The contents of eleven $0.5m^2$ quadrats were cropped over a depth range from 7.5m to 33m. Some of these samples were subsequently sorted, the algae oven-dried and weighed. The proportions contributed by species and groups to the total quadrat dry weight were calculated and are shown in Table 5.1. The contents of the remaining quadrats were dried and weighed and the total dry weight per square metre from all the quadrat data is shown in relation to depth in Figure 5.4. At 53m, four $0.5m^2$ quadrats were assessed visually for percentage cover by the species present and the means of these results appear in Table 5.2.

Two specimens each of the heavily calcified Pseudolithophyllum

Table 5.1. Relative contributions to the biomass at Ganzirri expressed as % of total dry weight per unit area sea floor.

Species or group	Depth (m) 15 No.1	15 No.2	21	29	30 No.1	30 No.2
Sphaerococcus	15	-	-	6	-	19
Phyllophora + Melobesioids	-	44	-	-	22	-
Phyllophora + Jania	34	-	-	-	-	-
Pterocladia + Jania	47	-	-	-	-	-
Cladophora + Jania	-	54	98	25	-	57
Cladophora	4	-	-	-	46	-
Ulva	-	2	-	10	8	11
Cystoseira	-	-	-	31	-	-
Miscellaneous Rhodophyta	-	-	-	27	21	-
Miscellaneous Phaeophyta	-	-	-	1	3	13

Table 5.2. Cover composition of underflora community at 53m, Ganzirri

	% cover
Pseudolithophyllum	46.5
Sand	35.0
Pseudolithophyllum (dead plants)	7.0
Peysonnelia	5.0
Ulva	2.7
L. ochroleuca (juveniles)	2.3
Halymenia	1.0
L.ochroleuca (adult holdfasts)	0.5

and moderately calcified Peyssonelia were decalcified with dilute hydrochloric acid to ascertain the relationships between area, dry weight, calcium carbonate content and organic matter content of their thalli. (This method has been successfully employed by Drew, 1973b, for decalcifying corals).

Table 5.3 Calcium carobonate and organic matter content (O.M.) of Pseudolithophyllum and Peyssonelia

	SLA cm^2g^{-1} dry wt	removed by acid ($CaCO_3$) % dry wt	residue (O.M.) % dry wt	corrected $CaCO^3$ content % dry wt	corrected[a] O.M. content %dry wt	SLA cm^2g^{-1} O.M.
Pseudo-lithophyllum	- 0.013±0.001	88.5±1.9	11.5±1.9	85.6	14.1	0.092
Peyss-onelia	- 0.068±0.003	67.9±0.9	32.1±0.9	59.9	40.1	0.170

a. Corrected by factor of 1.25 (cf. 1.33-1.47 in Table 5.5) for loss of soluble organic material to HCL.

The results, presented in Table 5.3. (above) show that 85.6% of the dry weight of Pseudolithophyllum and 59.9% of Peyssonelia consisted of carbonate material, the remainder being equivalent to organic matter. The decalcified dry weight of these species therefore is more comparable with the dry weights of non-calcified species such as Sphaerococcus. The SLA (specific lamina area = area of lamina per unit dry weight) is much higher when expressed on an organic matter basis. The values for biomass at 53m shown in Figure 5.4 were computed from percentage cover estimations using the SLA data in Table 5.3.

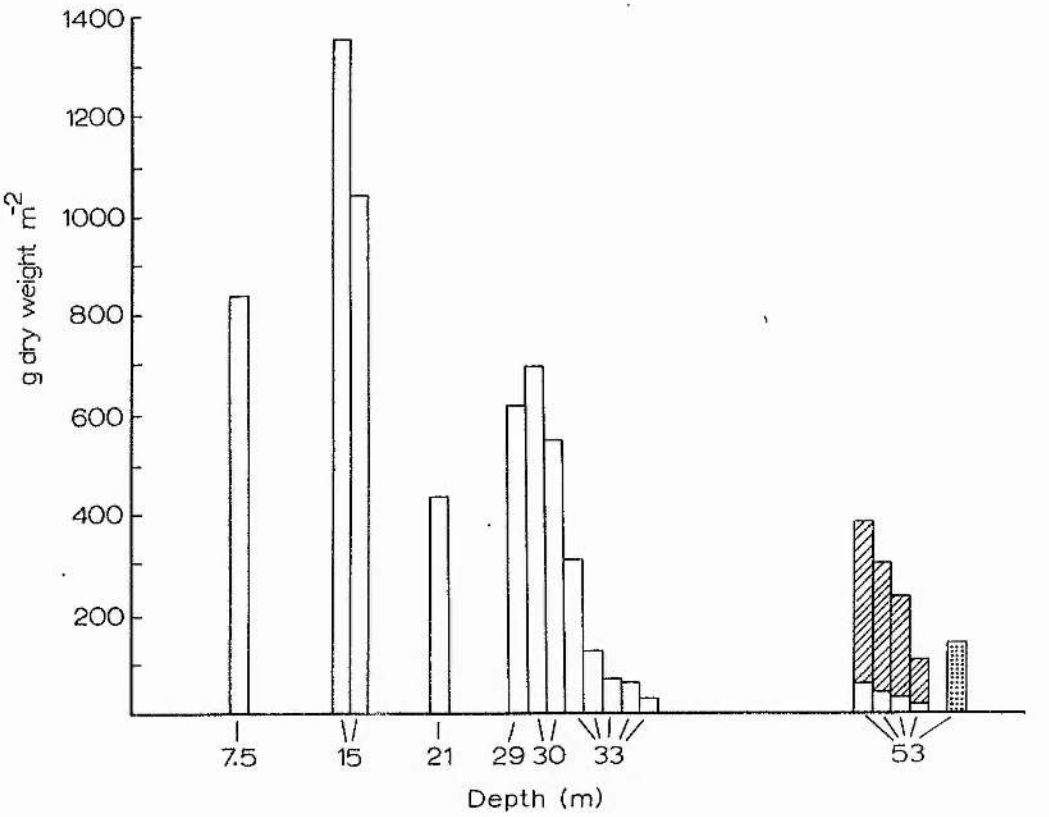


Figure 5.4. Total algal biomass collected by quadrat sampling at Ganzirri, September; hatched columns represent calcified fraction of deep samples (mostly Pseudolithophyllum); stippled column represents L.ochroleuca biomass. Depth scale approximate. Epidermis at 1.5m.

The greatest biomass (1360 gm^{-2}) was recorded at 15m where Cladophora, Phyllophora and Jania contributed substantially. Jania, a branched calcified species, was always found as an epiphyte, and separation from the host plant was not practicable. This species contributed about 50% of the dry weight of the host-epiphyte association. The standing crop (leaves only) of the Posidonia at 7.5, was 820 gm^{-2} whereas the algal biomass at 30m reached a comparable maximum of 690 gm^{-2} Minimum values of 36 and 52 gm^{-2} recorded at 33m indicated a decline in algal productivity with depth, which continued to the L. ochroleuca "forest" at 53, where the crop of non-calcified algae (including estimated biomass of decalcified Pseudolithophyllum) in the underflora was approximately 60 gm^{-2}. The sparse L. ochroleuca "forest" itself had a biomass in the region of 180 gm^{-2} (Drew 1974a).

d. Marsala - general description

A single experiment is reported (Chapter 7) which was conducted at San Teodoro close to Marsala at the western end of Sicily (Figure 5.1.). The area contrasts with Ganzirri in that the maximum depth within 1 km of the shore is 7.5m, and the sandy substratum is predominantly colonised by extensive beds of the angiosperm , Posidonia. Water temperature was 24°C. Peyssonelia , the alga used in the experiment reported grew in relative abundance on the undersides of boulders within 5m distance from the shore, at a depth of 2m.

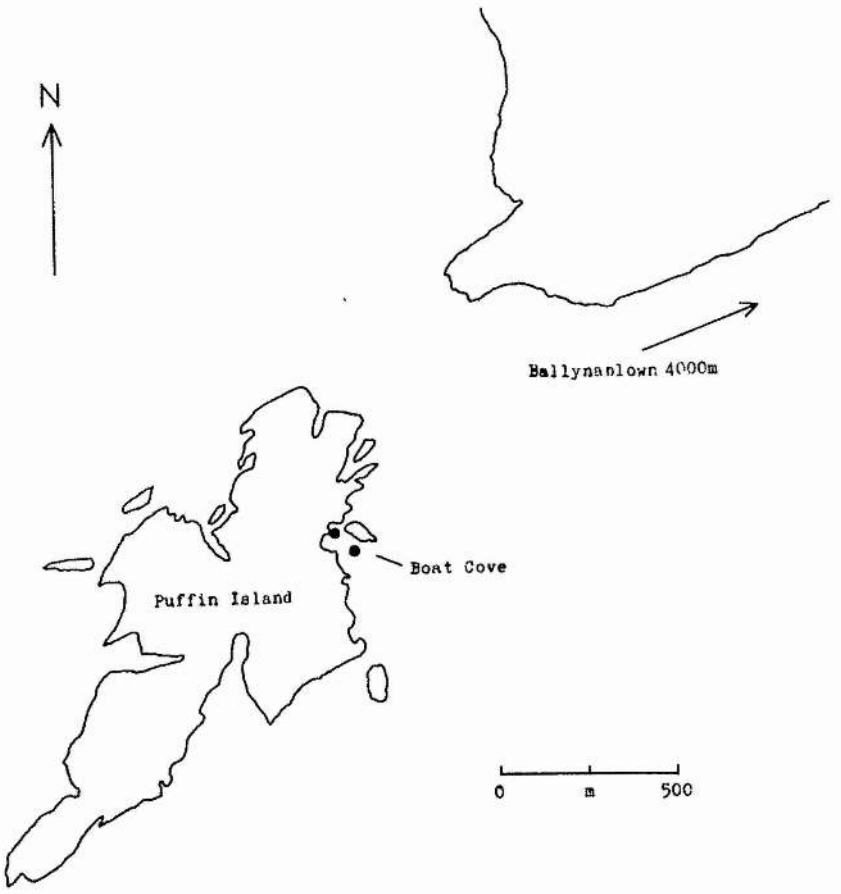
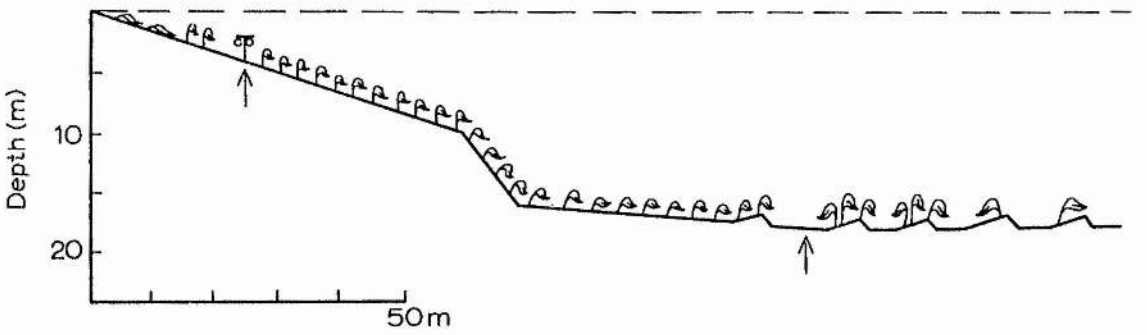


Figure 5.5. Map of Puffin Island showing experimental sites at Boat Cove, and the relative position of Ballynablown.



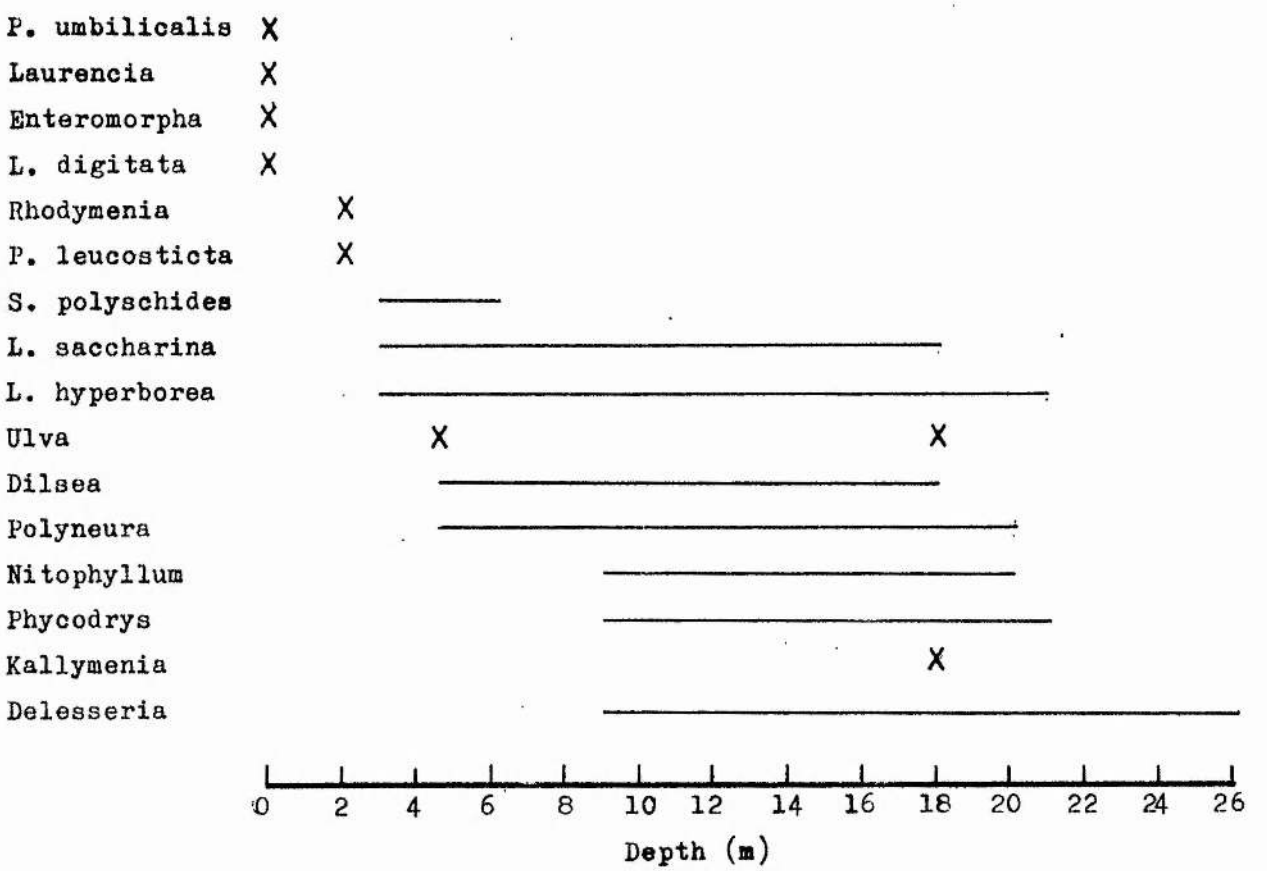


Figure 5.6. (upper). Profile of sea floor at Boat Cove, Puffin Island, showing dominant algae; , Laminaria hyperborea; , L.digitata. Arrows show positions of experimental sites.

Figure 5.7. (lower). Depth ranges of algae growing at Puffin Island in July.

B. British sites

a. Puffin Island - hydrology

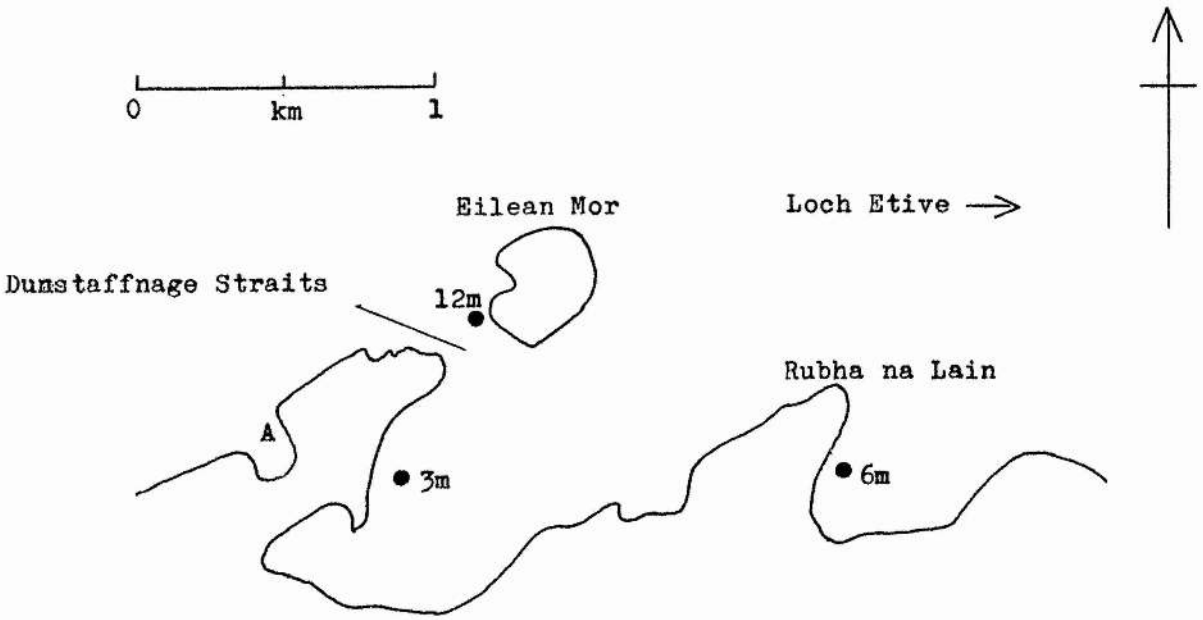
Puffin Island is an elongated outcrop of Old Red Sandstone, situated 400m off the coast of County Kerry, south-west Ireland (51°50'N, 10°20'W, Figures 1.2, 5.5). The south-east side of the island is very sheltered and the study area, Boat Cove, enjoys predominantly calm conditions during the prevailing westerly winds. The sea floor in Boat Cove, shown in profile in Figure 5.6. slopes gently with gravel and rock outcrops to a depth of 10m, then descends steeply as a rock face to 16m, sloping gently again to 18m where the bottom is rocky interspersed with large (10-20m wide) areas of sand and gravel. The slope from 18m to the maximum depth attained by diving, 27m, is extremely shallow and is an almost level rock and sand substratum. The tidal range at spring tides was 3.1m. Water temperatures were stable during July, the mean values being 13.0 $\pm$ 0.7°C at 3m and 11.9 $\pm$ 0.1°C at 18m. The profile in Figure 5.6. shows the site of the irradiance - depth transects reported in Chapter 4 (p108), and the positions of the two experimental sites.

b. Description of the flora

Puffin Island, in common with the rest of the south coasts of Ireland and England belongs to the transition zone between the warm temperate and boreal zones (Hedgpeth 1959), and the abundance of the warm-water brown alga Dictyopteris membranacea (stackh.) Batt. was indicative of the warm temperate influence. Although no quantitative studies were undertaken here, subjective assessment together with measurements of individual algal specimens indicated that the area was

perhaps the most productive in the present study. The depth ranges of the species used in the physiological experiments, and of other important members of the algal community as recorded on several survey dives, are shown in Figure 5.7. The sublittoral community was dominated by a forest of the brown algae Laminaria hyperborea (Gunn.) Fost. and Saccorhiza polyschides the latter penetrating no further than 6m while L. hyperborea forest continued to 20m while sparse individuals were recorded to 22m. The gravel areas at 18m supported a flora of young plants of Laminaria saccharina (L.) Lamour and large (∿50cm) adult specimens of Desmarestia dresnayi Lamour. ex Leman (not previously described in situ in Britain, Drew & Robertson, 1974b).

Of the species used in the experiments, Porphyra umbilicalis, Laurencia pinnatifida (Huds.) Lamour. (Both frequently a yellow-red colour due to exposure to high irradiance levels) and Enteromorpha linza were abundant intertidally. The rosy-red Porphyra lencosticta Thur. in Le Jol was collected in the upper sublittoral on the upper portions of L.hyperborea stipes, as was Rhodymenia palmata (L.) Grev. Dilsea carnosa (Schmidel) Kuntze grew on the rocks among the L.hyperborea boldfasts, and at shallower sites was frequently "bleached" to a yellow colour. At 18m, specimens were generally found prostrate on the gravel patches, attached to small stones. Ulva lactuca L. was seen only at 3-4.5m, below the L.hyperborea canopy, and at 18m growing with the same habit as Dilsea, on the gravel. Polyneura hilliae (Grev.) Kylin appeared at 4.5m where the thalli were frequently "bleached" to a greenish-yellow colour. The species reached its optimum depth at 15m, where an individual of extraordinary proportions was recorded, with a lamina area of 3200cm^2 (cf. Newton's 1931 range of 80-700cm^2). The lithophytic Delesseria sanguinea (Huds.) Lamour. reached its optimum depth between 15 and 20m. Specimens growing at the minimum depth of 8m were robust but



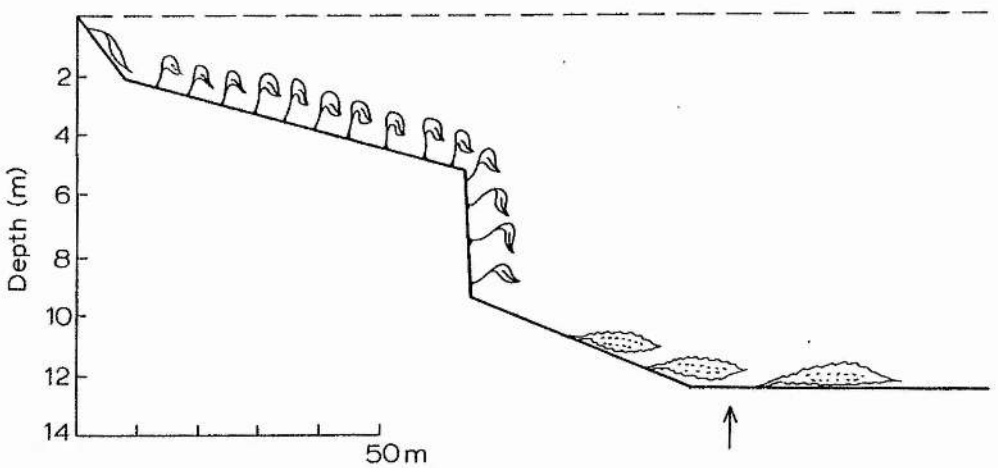


Figure 5.8. (upper). Location of experimental sites at Dunstaffnage. "A" marks collection site for Delesseria.

Figure 5.9. (lower). Profile of sea floor at Eilean Mor, Dunstaffnage, showing dominant algal species; Laminaria digitata; , L.hyperborea; , L.saccharina. Arrow shows position of experimental site.

showed damage from wave action. A maximum frond area of 200cm^2 was recorded at 18m. Delesseria, Cryptopleura ramosa (Huds.) Kylin ex Newton and Bonnemaisonia asparagoides (Woodw.) C.Ag. were the only macroalgae surviving at maximum depth studied, 27m. Nitophyllum punctatum (Stackh.) Grev. was present as an epiphyte on L.hyperborea stipes from 9m to 20m but much less abundant than Delesseria or Polyneura, taxonomically its close allies. The distribution and habit of Phycodrys rubens (L.) Batt. was similar to that of Nitophyllum but the population density at 18m was greatest in the former species.

Some experiments were conducted the following year, also in July, on the mainland, at Ballynablown (Figure 5.6.) where the sheltered nature of the cove produced essentially the same floristic pattern as that found at Puffin Island.

c. Dunstaffnage - hydrology

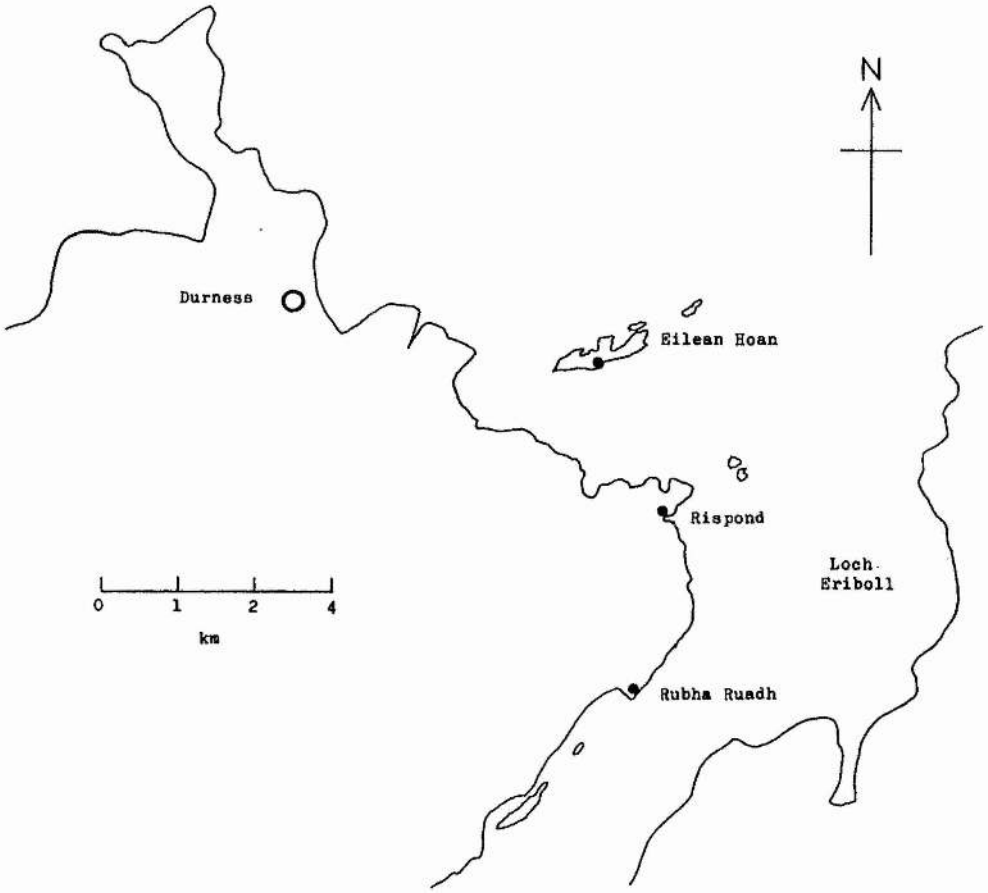
Figure 1.2 shows the position of the general area, and Figure 5.8 shows the relative positions of the experimental stations. Loch Etive, a long (25km) sea-loch, is a major influence on the hydrography. Only 3km from the study area are the noted Falls of Lora, where tidal influx and efflux produce currents of up to 750cm s^{-1} (15 knots). At the 12m deep experimental station in Dunstaffnage Straits, currents of up to approximately 150cm s^{-1} (3 knots) were encountered during spring tides.

Due to the large watershed of Loch Etive, the study area was frequently inundated by fresh water which formed a surface layer some 10-20cm thick during ebb tide. The layer was readily discernible by divers entering the water, when the differing refractive indices of fresh and saline waters produced distinct visual distortion at the interface of

the two layers. When present, this fresh or brackish water layer had to be avoided when filling experimental bottles at the surface (p.21). An unusual eddy system at Rubha na Lain (Figure 5.8) resulted in a high content there of water from Loch Etive. This was extremely brown in colour and reduced the light penetration, although this was not measured. The water temperature at the sites remained constant at 13.0°C at all three stations. The tidal range at springs was 4.1m.

d. Description of the flora

The sublittoral flora of Dunstaffnage Straits has been briefly described by Norton & Milburn (1972). A profile of the Eilean Mor station is shown in Figure 5. 9. Phycodrys rubens grew abundantly at 9m, epiphytic on L. hyperborea stipes, at the base of the cliff. Dilsea carnosa and Ulva lactuca were collected on the sand and gravel floor of the straits at 12m. As at Puffin Island, the gravel floor supported a population of L. saccharina and Desmarestia dresnayi; for the latter species, this was the first record at this site, and constitutes its northernmost known site (Drew & Robertson 1974b). Delesseria was not found at Eilean Mor, but abundant specimens were found on boulders in Dunstaffnage Bay (Figure 5.8) at a depth of 6m, which is very shallow for this species in unshaded conditions, and exposed plants were "bleached" to a green colour, although apparently healthy and actively growing. Plants and parts of plants shaded by other fronds or by stones appeared the "normal" red colour. At Rudha na Lain, Phycodrys rubens covered the boulder floor at a depth of 8m, and numerous plants of Dilsea carnosa were found among L. saccharina at 6m. The Dilsea specimens were extremely bleached, appearing a yellowish colour. Polysiphonia lanosa Tandy was collected



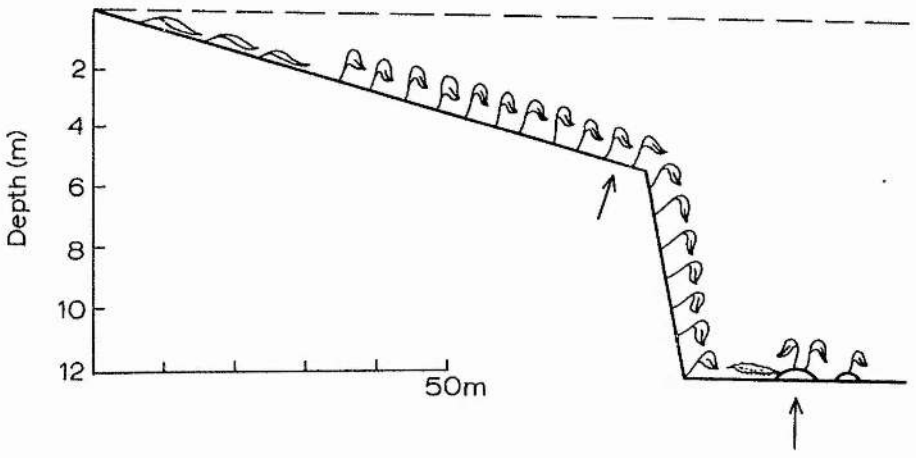


Figure 5.10. (upper). Map of Durness area showing sampling and experimental sites.

Figure 5.11. (lower). Profile of sea floor at Eilean Hoan, Durness, showing dominant algal species; ~, Laminaria digitata; ~, L.hyperborea; ~, L.saccharina. Arrows show position of quadrat samples.

growing epiphytically on Ascophyllum nodosum (L.) le Jol on intertidal rocks close to the 3m station. Shaded specimens were "normal" ruby red in colour but, again, more exposed plants were bright yellow.

e. Durness - hydrology

No prolonged series of in situ experiments was conducted at Durness ($58^{o}30'N$, $4^{o}40'W$) but algal material for surface experiments was collected at Rispond and Rubha Ruadh, shown in Figure 5.10, some in situ experiments were carried out at Rubha Ruadh and light attenuation transects were made at Rispond and Eilean Hoan, where biometric studies were also conducted. Although at spring tides, strong currents (100 - 200 ms^{-1}) sweep the north-west side of Eilean Hoan, the study site on the south-east side is relatively free from currents and is also sheltered from the prevailing westerly winds. The tidal range at springs at this site was found to be 4.5m. A profile of the study site is shown in Figure 5.11. A gradual rock slope from the intertidal region terminates abruptly at 5m depth, where a cliff descends almost vertically to the sandy boulder-strewn floor at 12-14m. The area in Figure 5.11 is the site of the irradiance - depth transects reported in Chapter 4 (p10θ.

f. Biomass data - Eilean Hoan site

Floristically, the area is a typical Laminaria hyperborea - dominated sublittoral profile (with some Saccorhiza polyschides), similar to Puffin Island and Dunstaffnage, as indicated in Figure 5.11. Two quadrats, each 0.5m^{2} in area were cropped (positions shown in Figure 5.11) one at 5m and one at 12m depth. At 5m, all L.hyperborea adult plants were

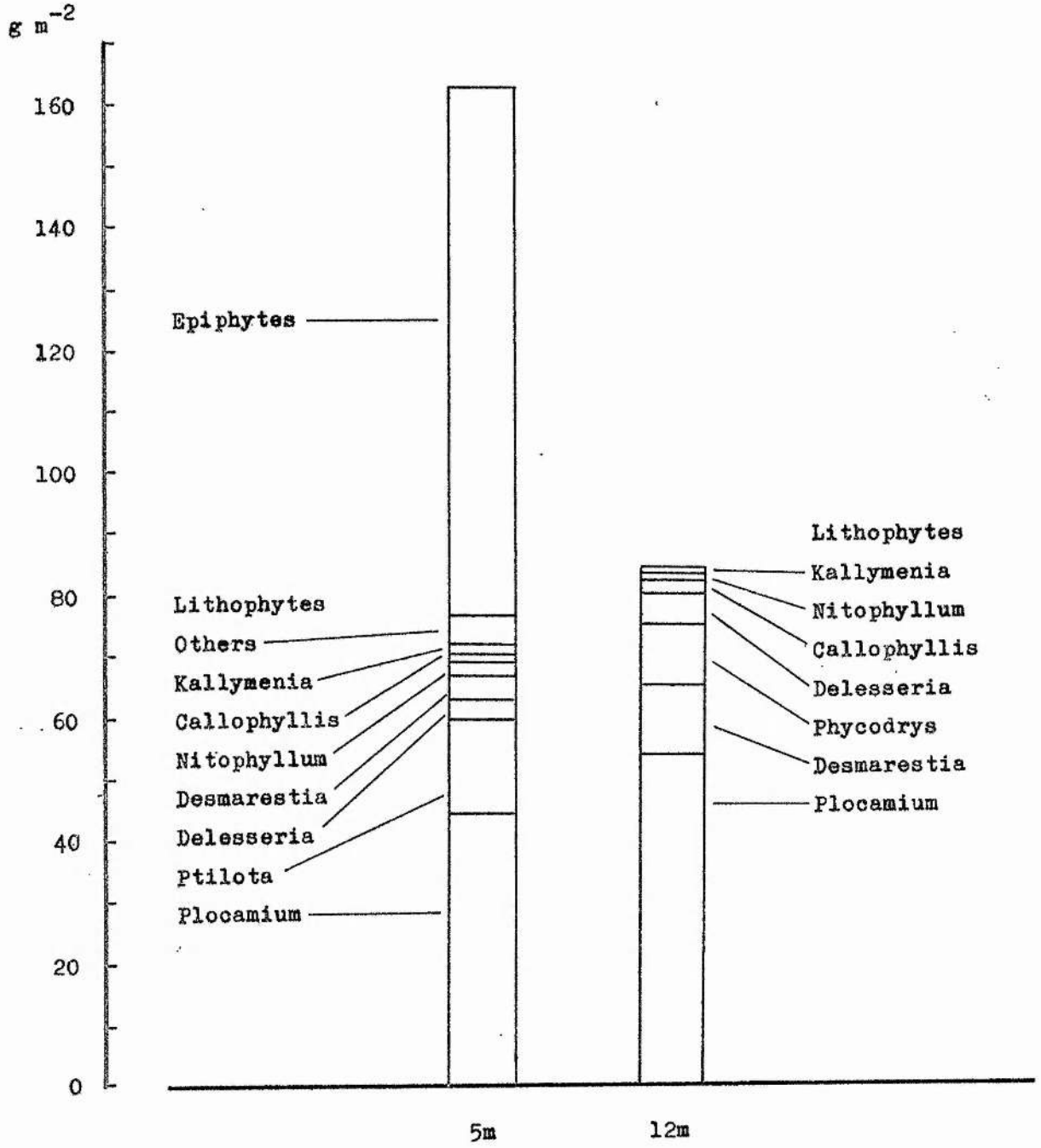


Figure 5.12. Non-laminarian biomass (g dry weight m^{-2}) at two depths at Eilean Hoan in August.

removed intact with epiphytes, and then lithophytic algae of the underflora were removed and placed in a nylon mesh bag. At the shore, all epiphytes were stripped from the host plants and oven dried together. The lithophytic algae were sorted according to species and dried separately. There were no epiphytes in the 12m quadrat, so L.hyperborea adults were not collected, only the lithophytes of the underflora. The dry weight values from the two quadrats are presented in Figure 5.12, converted to biomass per m^2. At 5m, epiphytes (86g m^{-2}) accounted for just over 50% of the total non-laminarian biomass of 162g m^{-2} (excluding a small amount of encrusting red algae which were not collected) which was almost twice the value of 84g m^{-2} attained at 12m. At 5m, the major part of the epiphytic biomass was contributed by Phycodrys rubens, but Ptilota plumosa (Huds.) C.Ag. was also important. The biomass of the lithophytic underflora community was slightly greater at 12m (84g m^{-2}) than at 5m (76g m^{-2}). At both sites, Plocamium cartilagineum (L.) Dixon contributed more than half of the total lithophytic biomass. At 5m, Ptilota accounted for 20% of the lithophytes but 12m was below the depth limit of this species. At 1m, 13% of the biomass was contributed by a single specimen of Desmarestia aculeata (L.) Lamour. which in fact occurred in relatively sparse numbers at this depth. Cryptopleura ramosa and Odonthalia dentata (L.) Lyngb. were subjectively assessed to be frequently important biomass contributors at 5m, but did not appear in the cropped quadrat.

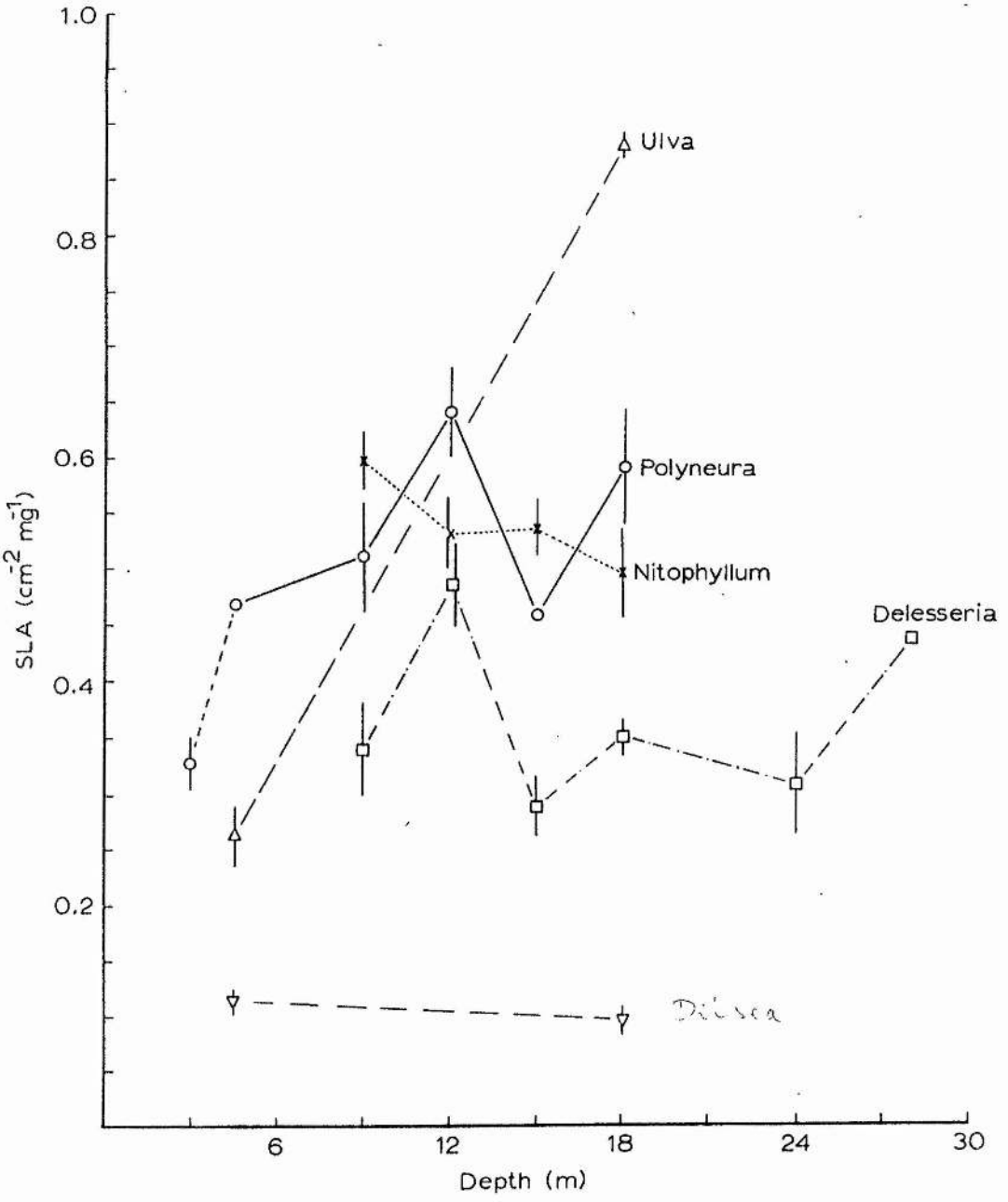


Figure 5.13. Variation of SLA with depth of growth, for several species at Puffin Island in July (4m material of Polyneura was from Ballynablown).

3. Some physiological parameters

a. Specific lamina area (SLA)

During the course of *in situ* experiments, specimens of algae were collected for determination of SLA (lamina area in cm^2 : lamina dry weight in mg as described in Chapter 2 (p.49). Determinations of SLA made for algae at Puffin Island are presented in Figure 5.13. No simple correlation with depth is seen except in the case of *Ulva* where the difference between the deep and shallow communities is highly significant. The summarised data for nine species of algae from different sites and habitats and sampled at different times of year, are presented in Table 5.4.

Table 5.4 Specific lamina area (SLA) of British algae, expressed on a dry weight basis.

Species	Month	Site	Depth m	n	SLA
Delesseria 0-20cm^2	April	Fife Ness	6	27	0.870 ± .046
0-20cm^2	July	Puffin I.	9-28	50	0.656 ± .037
0-30cm^2	"	" " "	9-28	50	0.559 ± .023
30-60cm^2	"	" " "	9-28	42	0.352 ± .015
>60cm^2	"	" " "	9-28	15	0.254 ± .021
Polyneura	"	" " "	18	5	0.590 ± 0.053
Nitophyllum	"	" " "	18	5	0.498 ± 0.044
Dilsea	"	" " "	18	3	0.099 ± 0.014
Porphyra umbilicalis	Dec	St Andrews	0	1	0.297
	Feb	" " "	0	2	0.325 ± 0.000
	Aug	Durness	0	2	0.446 ± 0.003
Plocamium	March	Fife Ness	6	2	0.100 ± 0.095
Laurencia	March	St Andrews	0	1	0.156
Ulva	July	Puffin I.	18	5	0.880 ± 0.013
	"	" " "	4.5	3	0.267 ± 0.013

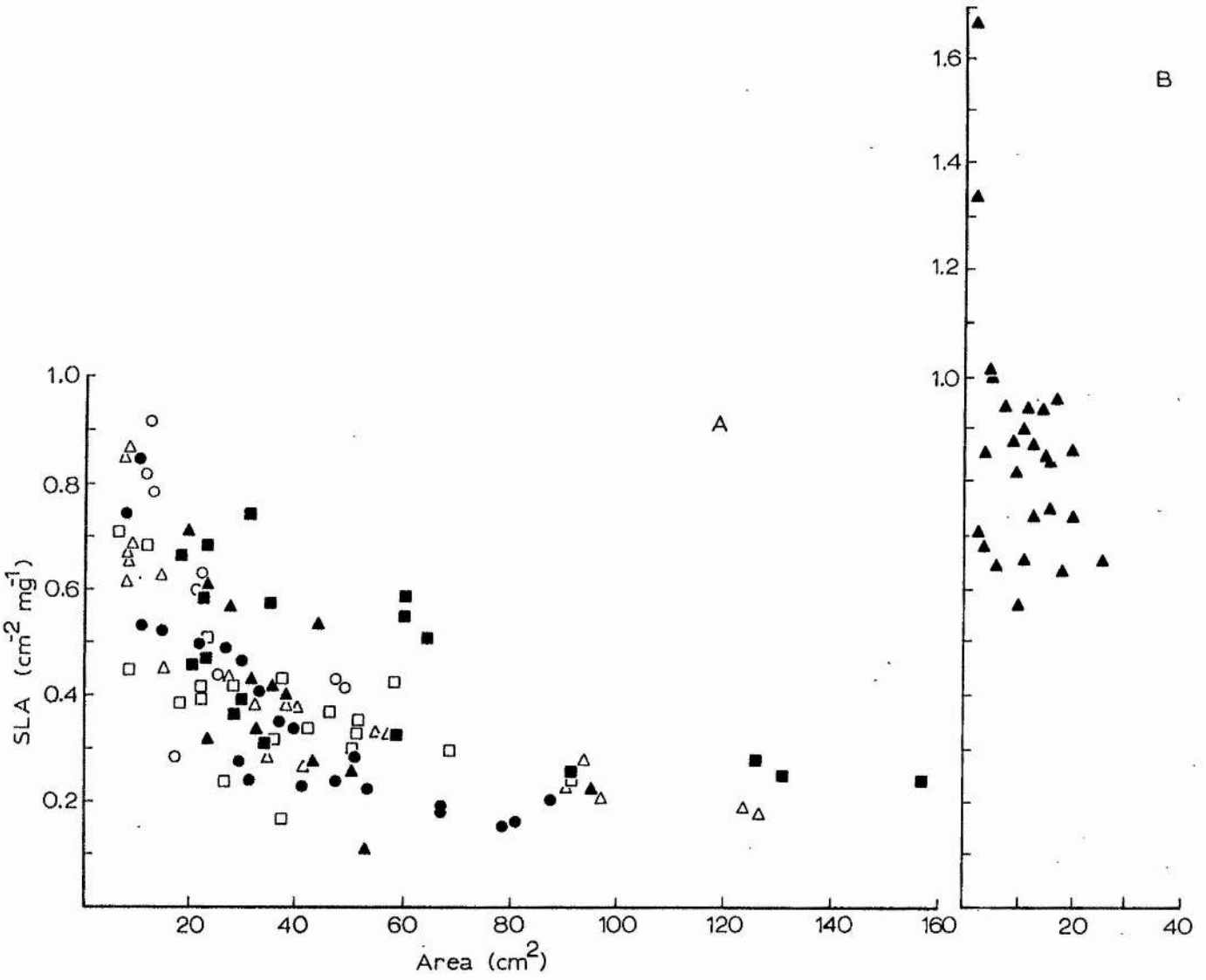


Figure 5.14. Variation of SLA of individual fronds of Delesseria, with respect to frond area. A, For several depths at Puffin Island, July; ▲ , 9m; ■ , 12,; ● , 15m; △ , 18m; □ , 24m; ○, 28m. B. For 6m at Fife Ness in March.

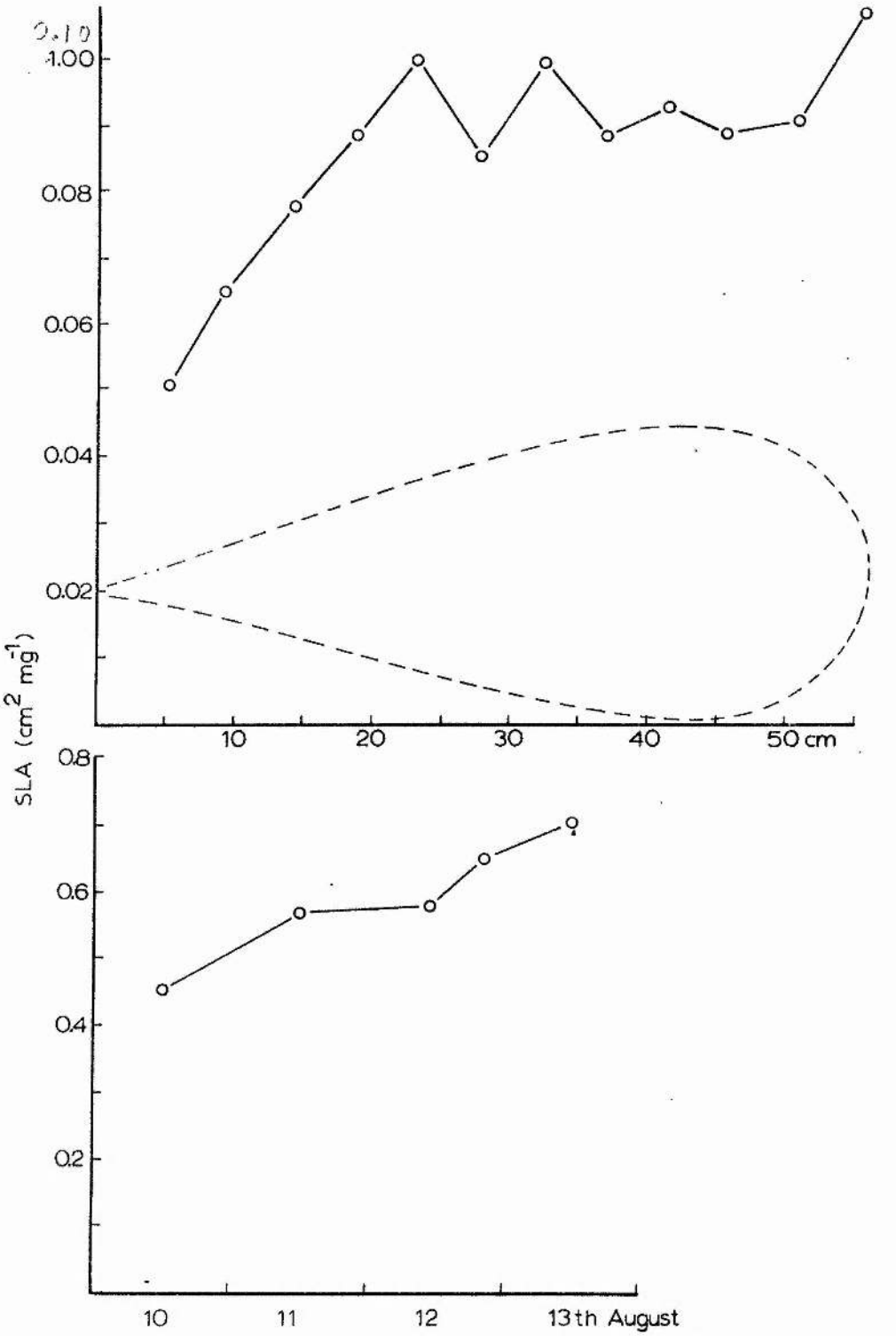


Figure 5.15. (upper). Variation of SLA with respect to position in frond in Dilsea (general outline of frond shown by dashed line).

Figure 5.16. (lower). Progressive increase in SLA of Porphyra kept in dark at 18°C for four days.

The sublittoral species of delicate form - Delesseria (smaller specimens), Polyneura, Nitophyllum and the deep form of Ulva - all had relatively high SLA values around 0.500 $cm^2 mg^{-1}$, while the shallow Ulva and Porphyra umbilicalis had values lower by half, around 0.300$cm^2 mg^{-1}$.

Considering the case of Delesseria in detail, SLA values for each individual "frond" or lamina of this species collected at 9, 12, 15, 18, 24 and 27m at Puffin Island and at 6m at Fife Ness, are shown plotted in relation to the area of the frond concerned, in Figures 5.14A and B. The Puffin Island values were markedly higher for fronds of small area, decreasing progressively with increase in frond area so that the maximum SLA was about four times the minimum. There was no correlation between SLA and depth. The mean value for the young, small fronds at Fife Ness in March was significantly higher than that for the older fronds of the same area class (0-20cm^2) from all depths at Puffin Island in July. This is the situation which would occur if the dry matter content of the fronds were higher per unit area or fresh weight as the season progressed. However, in Porphyra (see Table 5.4) SLA values increased as the season progressed, implying a decrease in dry matter content per unit area.

An investigation was made into the SLA of different regions of the thallus of Dilsea. A series of discs (2.3cm diameter) was cut along the median line from base to apex of a 50cm plant from 11m at Eilean Hoan. The SLA values of these discs were determined and are shown in Figure 5.15 in relation to their distances from the base of the plant. A steady increase in SLA from the initial low value of 0.051 $cm^2 mg^{-1}$ is seen up to 20cm from the base where a mean value of around 0.094 $cm^2 mg^{-1}$ is reached, rising at the most distal point to 0.108 $cm^2 mg^{-1}$.

Table 5.5 Increase in SLA after extraction by ethanol or Winkler reagents.

Species	Month	Site	Depth m	SLA $cm^2 mg^{-1}$ dry wt.	SLA $cm^2 mg^{-1}$ extracted dry wt.	Extracted / Unextracted
Delesseria	July	Puffin I.	18	0.209	0.409[a]	1.96
Dilsea	"	" "	18	0.100	0.167	1.67
Ulva	"	" "	4.5	0.250	0.583	2.33
Porphyra	Dec	St Andrews	0	0.297	0.394[b]	1.33
Ulva	"	" "	0	0.269	0.396	1.47

a. Extracted by 80% ethanol

b. "Extracted" by Winkler reagents.

Table 5.6 Specific lamina area (SLA) of Ganzirri algae, expressed on dry weight and alcohol extracted dry weight bases

Species	Month	Depth m	SLA $cm^2 mg^{-1}$ dry wt	SLA $cm^2 mg^{-1}$ extracted dry wt
Peyssonelia	Sept	53	0.068	0.213[c]
Pseudolithophyllum	"	53	0.013	0.118[c]
Ulva	"	53	0.464	1.111 ± 0.034[a]
	"	4.5	0.321[b]	0.767 ± 0.009[a]
	April	4.5	-	2.025
Porphyra	"	4.5	-	3.200[d]

a. based on eight replicates

b. calculated from $\frac{0.464}{1.111} \times 0.767$

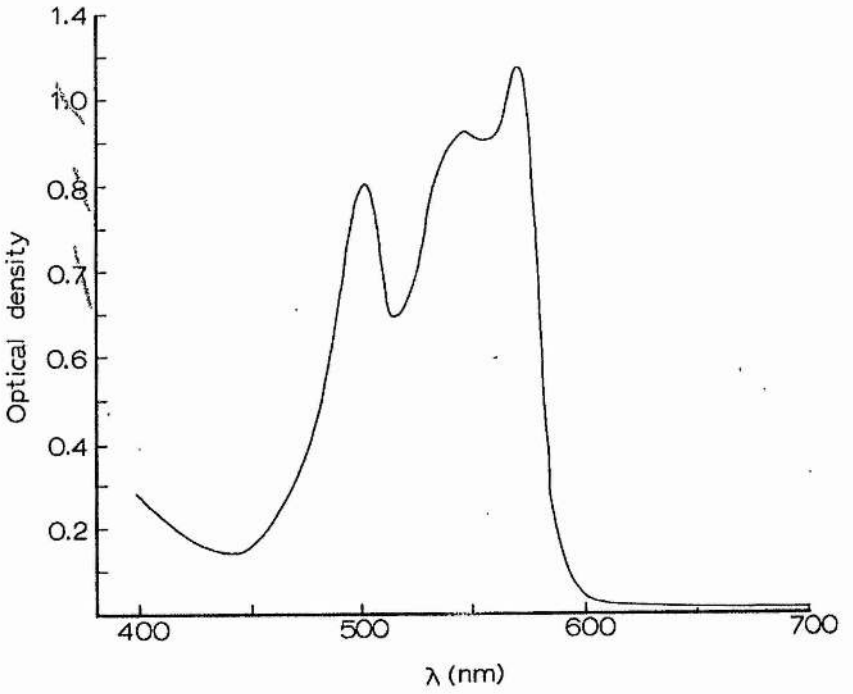
c. decalcified using HCL (see also Table 5.3)

d. based on an estimated 0.5mg extracted dry weight

The change in SLA in Porphyra when stored in the dark was measured to determine the effect, if any, of metabolic processes in modifying SLA. Tissue was collected from the intertidal at Eilean Hoan close to midday, and immediately samples were removed for SLA determination. The plants were then kept in black polythene bags in seawater at 18°C and further samples removed at intervals up to four days hence. Figure 5.16 shows that values rose constantly, the final value being 1.6 times the initial value, indicating a constant loss of dry weight per unit area.

Alcohol extraction procedures used in the ^{14}C method remove soluble carbohydrates and decrease the weight of solids per unit area of algal thallus. This process also occurs, to a limited and uncontrolled extent during exposure to strong alkali and acid in the oxygen technique used in the present work. Table 5.5 gives a comparison of SLA values for four species on unextracted and extracted dry weight bases using alcohol extraction and also after exposure to Winkler reagents. There was an increase in SLA of approximately 2-fold in former and 1.3-fold in the latter case.

Table 5.6 (see also Table 5.3) shows SLA values determined for five algal species at Ganzirri expressed on extracted (material from ^{14}C experiments) and unextracted dry weight bases. The ratio of extracted SLA to unextracted SLA of Ulva at 53m was 2.38, similar to that for Ulva at 18m at Puffin Island (2.33 Table 5.5). The heavily calcified Pseudolithophyllum had a very low SLA, but the SLA of Peyssonelia was close to that for Dilsea in Britain. The value of extracted SLA for Ulva in April was extremely high and is inaccurate due to the very low dry weights remaining (1-4 mg) after extraction The tissue of Porphyra disintegrated completely and could not be collected for weighing. In both species, the tissue involved consisted



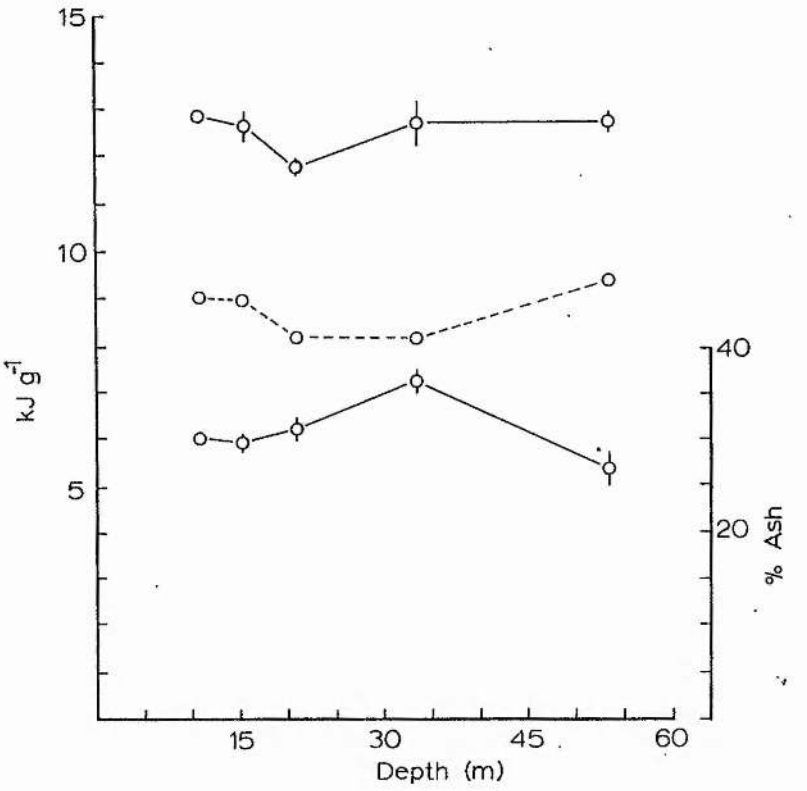


Figure 5.17. (upper). Absorption spectrum of R-phycoerythrin extracted from Delesseria at Puffin Island.

Figure 5.18. (lower). Variation of composition of Sphaerococcus with respect to depth of growth at Ganzirri in September; ash content as % of dry weight (lower curve); energy content per unit dry matter (broken curve); energy content per unit organic matter (upper curve).

of whole juvenile plants of about $3cm^2$ area.

b. Phycoerythrin content of normal and bleached tissue

Two specieswere studied, Delesseria at Puffin Island and Dunstaffnage, and Dilsea at Puffin Island only. / Delesseria of the "normal" red form was collected in shaded crevices at 6m and green material at the same site but growing in more exposed conditions (p.142). At Puffin Island, specimens of Delesseria were collected at 18m; some were extracted immediately and some were transferred to the shallow incubation platform at a depth of 3m where they were temporarily anchored and left for 24hr. After this period, the specimens appeared moribund, as indicated by patches of orange fluorescing tissue. Dilsea of the "normal" deep red colouration was collected at 18m and, at the shallower depth of 9m, yellowed specimens were obtained (p.140). Pigments were extracted as described in Chapter 2 (p. 50) and the aqueous extracts yielded absorption spectrum scans between 400 and 700nm as shown in Figure 5.17 for Delesseria from 18m at Puffin Island. All the extracts had identical absorption spectra, with the main absorption peak at 569nm and subsidiary peaks close to 545nm and 500nm, the three characteristic peaks of R-phycoerythrin (O'hEocha 1971). The concentration of phycoerythrin in each solution was calculated as a percentage (see p. 51) converted to µg phycoerythrin per mg extracted dry weight and the results are shown in Table 5.7. The values are also expressed approximately on an area basis by conversion using SLA values (from Tables 5.4 and 8.3). On a dry weight basis, the highest phycoerythrin content was found in normal Delesseria from 18m, whereas after 24hr pretreatment at 3m, this was reduced to one third. At Dunstaffnage, the

Table 5.7 Phycoerythrin content of algae with thalli of different colours

Species		Site	depth m	SLA $cm^2 mg^{-1}$	Phycoerythrin $\mu g\ mg^{-1}$	$\mu g\ cm^{-2}$
Delesseria	red	Puffin I.	18	0.350	40.6	115.9
" " "	red[a]	" "	18	0.350	15.7	44.7
" " "	red	Dunstaff.	6	0.202	23.9	118.3
" " "	green	" "	6	0.101	1.8	17.5
Dilsea	red	Puffin I.	12	0.100	23.0	230.4
" "	yellow	" "	9	0.100	2.0	19.5

a. moribund after 24h at 3m

Table 5.8 Ash and energy content of Sphaerococcus

Depth m	kcal g^{-1} dwt	kJ g^{-1} dwt	Ash %	kcal g^{-1} Org. Matt.	kJ g^{-1} Org. matt.
10.6	2.15	9.00	30	3.07	12.8
15.2	2.13	8.92	30	3.02	12.6
21.2	1.95	8.16	31	2.82	11.8
33.3	1.94	8.11	36	3.03	12.7
54.5	2.23	9.33	27	3.04	12.7

red material of the same species had a lower content on a dry weight basis than the Puffin Island material, but the green tissue had virtually no phycoerythrin. Red tissue of Dilsea at Puffin Island had a high content and about twelve times the amount found in the yellow material. On an area basis, the phycoerythrin contents of the Delesseria from both sites were very similar, and that of Dilsea was twice that of Delesseria.

c. Ash and energy content of Sphaerococcus growing at different depths

Samples of Sphaerococcus as used in the physiological experiments were collected, at Ganzirri, at five depths from 11 - 53m, at about midday. They were transported to the laboratory as quickly as possible to avoid undue respiratory loss, cleaned of adhering debris, then dried in an oven at 100°C. The samples were then transported back to Britain in aluminium foil packages for bomb calorimetry as described in Chapter 2 (p. 51). Two subsamples of each depth sample were used for each determination, except in the case of 11m where material was limited to one sample. The results are presented in Figure 5.18 and Table 5.8. The ash content showed a maximum value of 36% of the dry weight at 33m decreasing to the deeper and shallower sites. As might be expected, the values of energy content on a dry weight basis showed the opposite trend, reaching a maximum of 9.33 kJ g^{-1} (2.23 $kcalg^{-1}$) at 53, and a minimum of 8.11 kJ g^{-1} (1.94 $calg^{-1}$) at 33m. The more meaningful values of energy per unit organic matter (ash-free dry weight), showed that the variation in energy on a dry weight basis was largely due to variation in ash content. Thus, organic matter had a fairly constant energy content of

about 12.7 kJg^{-1} (3.03$kcalg^{-1}$) except for a low value of 11.8 kJg^{-1} (2.82 $kcalg^{-1}$) at 21m.

4. Taxonomical comment

During the initial part of the study, specimens of Ulva collected from all depths at British sites were considered to be U.lactuca L. It was noticed however that whilst shallow specimens (less than 10m deep) were invariably tough in texture and "grass-green" in colour, the deeper ones (10.18,) were more delicate, and of an "olive-green" hue. These differences were quantitatively supported by the differential in SLA values between specimens collected shallow and deep which was ascribed to a phenotypical response to "sun" and "shade" environmental conditions. Detailed examination of a limited quantity of material from Puffin Island, however, revealed the presence of minute marginal "spines" on shallow material, leading to their classification as U.rigida C.Ag. (J. Price pers. comm.). The thallus forms then corresponded to the comments of Papenfuss (1960) that U.rigida was "leathery and rigid". While it is tentatively suggested here that the tendency for U.rigida to occur at shallow sites and U.lactuca at deeper ones is a general one in Britain, fairly intensive sublittoral studies (Kain 1960, 1961; McAllister et al. 1967; Norton & Milburn 1972) and littoral ones (Russel 1968) recorded only U.lactuca. Dixon (1961) however found U.rigida at the Channel Islands although no site of growth was given, and Norton (1976) recorded both species from the littoral and sublittoral on three east Scotland sites.

Turning to the situation in the Mediterranean, Ulva specimens again were all ascribed to U.lactuca, although morphological differences similar to those described in British specimens were once more noted. In a study

of Ulva from the Atlantic and the Mediterranean (Naples), Föyn (1955) found no interfertility, and ascribed Mediterranean material to a new species, U.thuretii . He considered that only one species was present at Naples, but Giaccone (1972) recorded two at Ganzirri; one, U.lactuca, probably equivalent to U. thuretii and the other, from deeper habitats, U.olivascens Dangeard. It seems possible, considering the specific epithet of the latter, and from the olive colour noted in deep specimens used in this study, that these belong to U.olivascens.

These notes are offered in the spirit of Lewin's (1974) plea to physiologists to be as specific as possible about the identity of their experimental material, and his criticism of algal workers especially, in drawing general phyletic conclusions from work on a small number of ill-defined species.

It is suggested that the nomenclature of the Rhodophyta studied here is somewhat less confused than that of the genus Ulva and that the identifications made conform to those of other workers.

5. Discussion

a. Flora and biomass

Since the early observations of Oltmanns (1892) and later studies of Shelford & Gail (1922) and Kitching (1941) a correlation has been noted between the reduction of algal growth with increase in depth in the sea and the concomitant attenuation of light by the seawater. More recent workers (Kain 1960, 1961; McAllister et. al. 1967; Norton 1968) noted that the nature of the substrate underwater was another major factor. Degree of exposure was also a factor obviously associated with species distribution and has been quantitatively studied by Jones & Demetrapolous (1968) and Doty (1971)

In the present study, at Ganzirri, the rock substrate was colonised with 100% cover to all depths studied (maximum 60m) indicating that ambient light was sufficient for algal growth. However due to the growth habit of the deep-growing calcareous Pseudolithophyllum, sand patches at 53m were not colonised, as they were at 30, where erect species such as Cladophora, Phyllophora and Sphaerococcus thrived on all substrates. Giaccone (1972) noted that L.ochroleuca was present on bedrock only and absent in areas of of clastic sediments and gravel areas of particle size less than 5mm diameter. At 27m at Puffin Island, although rock substrate was available, very few algae were present, and in this case it can be concluded that the colonisation of algae was limited by low irradiance.

The flora recorded at 50-60m at Ganzirri was similar to that reported by Molinier (1960) for "les fonds corralligènes" at Corsica, including Pseudolithophyllum expansum and Peyssonelia spp. at about 80m depth. The flora described by Larkum et. al. (1967) at Malta was predominantly composed of the erect (i.e. non encrusting) calcareous green algae Udotea petiolata and Halimeda tuna, although Peyssonelia squamaria was also present. The absence of these non-encrusting forms as important members of the Ganzirri flora must be considered one of the most significant differences from other warm water floras, for Molinier (1960) also cites "numerous shade algae" namely Udotea spp. and Halimeda spp. in the "fonds precoralligènes" at 50-80m at Corsica. The absence of non-encrusting forms at deep sites at Ganzirri has been ascribed by Giaccone (1972) to the increased scouring effect of the currents at greater depths. The same author stated that the presence of a species as large as L.ochroleuca at such depth was attributable to the breaking down of boundary layers at the "living surface" and that due to this, the algae are able to maintain luxuriant growth due to heterotrophic uptake of the plentiful micronutrients. There seems to be no direct evidence to support this speculation, indeed,

inorganic micronutrients would not require heterotrophic uptake.

The communities observed at the British sites were similar to typical L.hyperborea-dominated sublittoral communities described by previous authors (Kitching 1941 ; Kain 1960, 1961; Norton & Milburn 1972; McAllister et al 1967; Smith 1967). The position of L.hyperborea in the British sublittoral is a much more influential one than that of L.ochroleuca At Ganzirri. At Ganzirri there were 0.5 adult plants per m^2 (Drew 1972b) with a lamina area index(LAI = lamina area per unit substrate area) of approximately $0.5 m^2 m^{-2}$ whereas at Eilean Hoan the LAI of L.hyperborea was $7 m^2 m^{-2}$ (At Arisaig, Argyllshire a maximum of $12.5\ m^2 m^{-2}$ was recorded - Robertson unpublished) for a density of 16.5 plants m^{-2}. It is likely that the shade created by this latter canopy (p.113) influences the colonisation of the underflora much more than L.ochroleuca at Ganzirri does.

Table 5.9 shows biomass data from the Ganzirri study compared with data of other authors. Although the Posidonia crop at 7.5m at Ganzirri was close to that recorded at Malta (Drew & Jupp 1976) the algal biomass at 15m at Ganzirri (predominantly red algae) was almost ten times that at this depth at Malta (predominantly brown and green algae, Larkum et al. 1967). Although the biomass at 53m at Ganzirri was four times that found at the same depth at Malta, 80% of this was composed of adult L.ochroleuca and so the underflora was in fact about the same as the crop at Malta, despite the absence of erect forms.

Table 5.10 gives a comparison of some estimates of sublittoral biomass in the temperate zone. The L.hyperborea standing crop measured by Drew at Eilean Hoan was similar to that recorded for other Scottish sites (Robertson 1970), indicating that this site was fairly typical. The values recorded by Bellamy & Whittick (1968) for non-laminarian algae were similar to those recorded in the present work. The proportion

Community	Site	Depth m	Biomass g m^{-2}	Comments	Author
Posidonia	Ganzirri	7.5	850	Dry wt.	Present study
Phyllophora, Pterocladia Jania, Cladophora	" "	15	1200	partly calcified dry wt.	" "
L.ochroleuca	" "	53	142	dry wt.	" "
underflora	" "	53	39	decalcified dry wt.	" "
Posidonia	Malta	7.5	900	dry wt.	Drew & Jupp (1976)
Sargassum	"	15	138	dry wt.	Larkum et al. 1967
Halimeda	"	53	46	decalcified dry wt.	" "
Cystoseira	-	1	2583	dry wt.	Bellan-Santini (in Drew 1971)

Table 5.9. Biomass of some sublittoral communities in the Mediterranean Sea

Table 5.10 Biomass of some sublittoral communities in temperate waters.

Community	Site	Depth m	Biomass g dry wt m^{-2}	Comments	Author
L.hyperborea	Eilean Hoan	3	4368		Drew (pers.comm.)
" " "	" "	15	645		" " "
" " "	Arisaig	3	5050	annual maximum	Robertson (1970)
underflora	Eilean Hoan	5	162	epiphytes + lithophytes	Present study
" " "	" "	12	84	lithophytes only	" " "
	Berwickshire	1-3	265	epiphytes only	Bellamy & Whittick (1968)
	" "	10-12	63	" "	" " "
Macrocystis	California	0-20	10000		Aleem (1972)
underflora	" "	8	1385	Corallines, decalcified	" "
Chondrus	New Brunswick	2	1231	" "	Taylor (1972)
Porphyra	Japan	0	140	cultivated "nori"	Satomi et al. (1967)

of total plant biomass supplied by non-laminarian species at Eilean Hoan was around 4% at 5m and 11% at 11m. Bellamy and Whittick found that epiphytes could account for 9% of the crop at 3m. Kain (1976) found that (on a fresh weight basis) the rhodophyte proportion of a sublittoral community decreased from 12.4% (2.6% lithophytes + 9.8% epiphytes) at approximately 4m (below mean sea level) to 7.6% (2.7% epiliths + 4.9% epiphytes) at 7m. These results agree with the present findings notably in that although the epiphytic community decreased with depth, the lithophytic biomass remained relatively constant. The disappearance of the epiphyte community at 12m at Hoan can be explained in terms of substrate limitation. Drew (pers.comm.) found a density of 16.5 canopy plants per square metre at 3m depth at Eilean Hoan and 6.4 at 15m. Assuming a mean stipe diameter of 2.5cm and length of 150cm for these plants, this gives a surface area of

$$\pi dh \times n = 3.142 \times 2.5 \times 150 \times 16.5 \simeq 19400 cm^2 \text{ at } 3m$$

and

$$\pi dh \times n \simeq 3.142 \times 2.5 \times 75 \times 6.4 \simeq 3800\ cm^2 \text{ at } 15m$$

Thus, adding $1m^2$ to each, for original rock substrate, the total colonisable substrate for non Laminarian species will be 2.94 m^2m^{-2} at 3m, and 1.38m^2m^{-2} at 15m, assuming that, although the holdfasts of L.hyperborea may actually cover around 20% of the rock at 3m, they are still available for colonisation by smaller algae. These figures are equivalent to the dimensionless "surface index (S.I.)" of Dahl (1973) defined as the ratio of colonisable surface area to the area of a flat plane with similar boundaries (i.e. in "plan view"). They compare with the overall figure of 3 quoted by this author for a coral reef area, but for very small algal forms, the effective figure can be as high as 15. Clearly, the number will always have a value equal to or greater than 1, and its reduction in value with increased depth, due to the reduction in density of L.hyperborea in

British waters, may be a prime influence on the biomass of the underflora.

Odum (1959), on the question of dominance in plant communities states that a dominant species exerts "a major controlling influence on the community","generally has largest production", is frequently "part of the overstory" and dominance is usually attributable to "a very small proportion of the species present". In all these respects, L. hyperborea at the British sites studied was a classically dominant species. At Ganzirri however, although L.ochroleuca had a high production and formed a conspicuous overstorey, it was unlikely that the species substantially influenced the community (e.g. by shading, p.122). Although it is possible to regard the diversity of species of the Rhodophyta in British waters as part of the general "success" of this division accorded by the theory of chromatic adaptation (Rabinowitch 1945), it is more reasonable to regard the predominantly red algal understorey as the diverse non-dominant part of the sublittoral community. Even although red algae do predominate at the limits of the photic zone in British waters, in terms of biomass of the whole sublittoral profile, it is unlikely that the red algae frequently exceed the 5-10% level found in the L.hyperborea forest itself.

b. Specific lamina area

Although wide variations were recorded between SLA values of specimens of algae studied at various depths, a simple relationship with depth was seen only in Ulva (to some extent in Polyneura), for which a taxonomical explanation has been tentatively advanced (p.152). Two variables can be regarded as being significantly affected by depth, namely irradiance (Chapter 4) and water movement (Chapter 3). Light is an established factor in influencing SLA changes in higher plants, the general finding being that "sun" plants have thick leaves of low SLA and

"shade" plants have thin leaves of relatively higher SLA (Gabrielsen 1948; Rabinowitch 1951; Bjorkman and Holmgren 1963). This increase in SLA at low irradiance can be regarded as an adaptation to increase light absorption per unit leaf weight. Spence & Chrystal (1970b) showed that the SLA of leaves of freshwater macrophytes could increase up to three-fold with increasing depth of growth, depending on the attenuation characteristics of the water. In marine macroalgae, Robertson (1970) and Jupp (1972) showed a 2-fold difference in SLA of L.hyperborea fronds from 3m and 9m (.050 $cm^2 mg^{-1}$ and 0.100 $cm^2 mg^{-1}$ respectively, in the most significant case). As suggested by Coombe (1966) for vascular plants, SLA may vary in accordance with factors other than irradiance, and in the marine environment, turbulence has frequently been isolated as being important in this connection. Norton & Burrows (1969) noted differences in frond structure and growth between areas of turbulence and of calm water. More specifically, Norton (1969) found that fronds of Saccorhiza polyschides from turbulent areas were thicker (implying low SLA) than those from still water where the fronds were "cucullate" (i.e. hood or cape-shaped) and thin. Similar sheltered water forms have been noted in L.hyperborea and L. digitata and were sufficiently distinct to be originally accorded species status as L.cucullata, now, however, reduced to forma status. (Svendsen & Kain 1971). This evidence that algal SLA might be influenced by water movement was strongly supported by the work of Larkum (1972), who found that increase in depth resulted in higher SLA of L.hyperborea and this was considered to be due to reduced wave action which had a similar effect, independent of depth. Importantly, Larkum found that increase in SLA was occasioned by a decrease in number of cell layers,

which is the mechanism for this process in vascular plants (Björkman & Holmgren 1963). With the exception of Dilsea, however, the "laminar" algae used in the present study had either mono- or di-stromatic sheet-like thalli with no scope for change in thickness by altering the number of cell layers. The lack of such gross changes in the simpler plants used in the present study may represent a lack of flexibility on their parts. The only species with a thallus structure capable of adaptation by altering cell-layer number is Dilsea, and although some variation of SLA was noted, this was not of the same order as found in the other species (see Figure 5.13). The very significant decrease of SLA with increased area, shown by Delesseria (Figure 5.14) corresponds to the continuous enlargement of the midrib in this species, due to cell increase by cortification. The significant difference found between Delesseria SLA measured at Fife Ness in April and Puffin Island in July could be geographical (as was found in L.hyperborea by Larkum, 1972) but is more likely to be seasonal, suggesting a progressive build-up of storage materials as the season progresses. The influence of storage compound content on SLA was shown in Figure 5.16, where "starved" Porphyra showed a 1.55-fold increase in SLA over fresh material. The relative content of storage materials may be the main cause of SLA variation in the unspecialised mono- and di-stromatic species of algae.

As regards methodology, these findings emphasise the need for expediency in the sampling procedures used in measuring SLA. Also, variation in SLA of Dilsea was noted when material was removed from different regions of the same frond (Figure 5.15). This has been noted in L.hyperborea by Larkum (1972) and may correlate with corresponding differences in metabolic activity (Kain et al. 1976; Johnston et al., in press) emphasising the importance of selecting material from similar regions of the same frond for use in physiological experiments.

c. Phycoerythrin content

The results clearly corroborated the visual impression that the green form of Delesseria at Dunstaffnage and yellow Dilsea at Puffin Island were due to reduced phycoerythrin content compared with red material. A reduction of phycoerythrin content in discoloured red algae growing in the littoral zone is well known (Fritsch 1945, p409). Brody & Emerson (1959) showed that although blue and green light (i.e. of wavelengths absorbed by phycoerthrin) of low irradiance (0.01 mW cm^{-2}) would stimulate phycoerthrin production in the red unicell Porphyridium, at irradiances above 1.0mW cm^{-2}, the content declined. Similarly, it has been shown that in the shallow-growing Mediterranean macrophytic red species Petroglossum nicaense and Gracilaria compressa the phycoerythrin content of the exposed distal parts of plants was much lower, and the carotenoid content was correspondingly higher, than proximal shaded parts (Calabrese 1972; Calabrese & Felicini 1973). The phycoerythrin content is, however, also dependent on nitrogen availability (Calabrese & Felicini 1970) due to its protein nature. In terms of chromatic adaptation, since reduction of phycoerythrin occurs at relatively high irradiances, this loss of the green-absorbing pigment is less critical, since if irradiances are high enough to bleach, the spectral quality of the irradiance will not yet be sufficiently monochromatic to preclude effective absorption by chlorophyll. Algae with reduced phycoerythrin contents can photosynthesise adequately, presumably utilising their chlorophylls and carotenoids (Calabrese & Felicini 1973; this thesis, Chapter 7 p.219).

In absolute terms, the pigment contents shown in Table 5.7 were higher than those of around 12 µg phycoerythrin mg^{-1} dry weight found for the shallow Petroglossum naicaense, Pterocladia capillacea and Gracilaria compressa (Calabrese & Felicini 1970, 1973; Calabrese 1972) but much

lower than the values of 300 µg mg^{-1} found for the massive tropical rhodophyte, Eucheuma isiforme from 10m depth at Florida (Moon & Dawes 1976). This latter figure is extremely high, however, compared with the total pigment content of 10-20 µg mg^{-1} which is usual for terrestrial plant leaves (Bjorkman & Holmgren 1963).

The reduction in phycoerythrin content of Delesseria when transferred to a shallow depth for only 24 hr is a lethal effect, as shown by experiments in Chapter 7, but presumably proceeds by the same mechanisms as in the apparently healthy "bleached" specimens of the littoral species Laurencia and Polysiphonia (p.142) or of the sublittoral Dilsea and Delesseria growing near their upper depth limits, described above. The gradual increase in irradiance levels from winter to summer may allow the less susceptible chlorophylls and caroteniods to increase in content as the content of phycoerythrin is gradually reduced by the higher irradiance.

d. Sphaerococcus - Ash and energy content with respect to depth

In terrestrial plants, ash content is frequently only 5% (Westlake 1963) but in marine plants the proportion is consistently higher. Paine & Vadas (1969) found a range of 12% in Endocladia muricata to 83% in the calcified Lithothamnion sp. This latter value compares closely with the estimate of 88.5% calcium carbonate content found in the present work for the similar genus, Pseudolithophyllum. Detailed studies of the effects of depth on the ash content of individual species are not available, but seasonal studies reveal that ash content varies with physiological state. Himmelmann & Carefoot (1975) showed that the ash content of red, brown and green algae was lowest in summer, correlating inversely with stored carbohydrate content. It thus seems strange that

the ash content of Sphaerococcus should be at a maximum at 33m, the apparent optimum depth for this species. A possible explanation is that contamination of the 33m material by calcareous algae (Melobesoids) and animals (bryozoans) may have resulted in an anomalously high ash content.

The energy content of about 9kJ per gram dry weight (2.2kcal g^{-1}) is very low compared with values for terrestrial plants of 14-18Jg^{-1} (3.3-4.3 kcal g^{-1}) (Šesták et al. 1971, p.370). Again,the low values for seaweeds are largely due to the high ash content, and the value for Sphaerococcus is very similar to that of 8.8 kJg^{-1} (2.1 kcal g^{-1}) found for Polysiphonia subulifera growing at 30m at Malta (Larkum et al. 1967).

The energy content of the ash-free dry weight gives some insight into the composition of the organic matter component of the plant. Westlake (1963) gives values of 15.5 kJg^{-1} (3.7kcal g^{-1}) for glucose, 17.6 kJg^{-1} (4.2 $kcalg^{-1}$) for starch and cellulose, 23.9 kJg^{-1} (5.7 kcal g^{-1}) for crude protein and 39.8 kJg^{-1} (9.5 kcal g^{-1}) for fats. The very low values obtained for Sphaerococcus,around 12.5 kJg^{-1} (3.0 kcal g^{-1}) indicate that the organic matter must be substantially composed of carbohydrate. The values are comparable to those of 12.1 kJg^{-1} (2.9 kcal g^{-1}) and 11.3 kJg^{-1} (2.7 $kcalg^{-1}$) for Polysiphonia subulifera and Peyssonelia squamaria in Malta (Larkum et al. 1967). Values for temperate algae seem to be higher however (Paine & Vadas 1969; Robertson 1970; Jupp 1972; Himmelmann & Carefoot 1975) and centre around 19.5 kJg^{-1} (4.65 $kcalg^{-1}$) in the Rhodophyta. Paine & Vadas (1969) regarded the figures produced by Larkum et al. (1967), and therefore of the present study, as "too low to be biologically realistic", but it seems that the low values may be a characteristic of warm-water algae. In view of the

overall low nature of the Sphaerococcus values, the slight reduction in energy content of organic matter at 21m is not sufficiently significant to merit further speculation.

In summary, it can be said that in Sphaerococcus, over a considerable depth range (40m) the energy content on an organic matter basis is constant at about 12.5 kJg^{-1} (3.0 $kcal\ g^{-1}$). The ash content appears to show a maximum, and therefore organic matter has a minimum, at 33m, which is near the optimal depth for this species.

This relative constancy of composition with respect to depth correlates with the finding that the majority of the red algae studied did not show a significant change in gross thallus structure (measured by SLA) in relation to depth.

CHAPTER 6

Photosynthesis measured in situ at depths down to 60m

CONTENTS

1. Introduction

An interest in *in situ* experiments on macroalgal photosynthesis was first generated by Englemann's (1884) chromatic adaptation theory which suggested that the different action spectra exhibited by the three major marine algal divisions (Chlorophyta, Phaeophyta, Rhodophyta) were correlated with the transmission spectra of the different types of seawater. Early and quite comprehensive studies by Gail (1922), Tschudy (1934), Printz (1939) and Levring (1947) utilised algal material collected by dredging and incubated at various depths in bottles suspended on a rope. The method was satisfactory particularly in respect of the large number of depths used, but unsatisfactory in the necessity of bringing algae to the surface for procedures in the boat, and the uncertainty of the exact depth source of the algae. The present method enabled the experimenter to observe the plants at their growth site and select specimens which were normal and healthy representatives of the species and community at that depth. Experiments using diving methods to study *in situ* photosynthesis have been carried out on marine macroalgae by Drew (1966), Drew & Larkum (1967), Drew (1972b), Drew et al.(1976) Johnston & Cook (1968), Johnston (1969) and Jupp (1972), on marine angiosperms by Drew & Jupp (1976) and on freshwater macrophytes by Wetzel (1965), Campbell (1972) and Campbell & Spence (1976).

It is the purpose of in situ experiments to investigate the actual natural process of photosynthesis as related to observed growth in the field, rather than the theoretical capabilities of the process as revealed by laboratory experiments. Laboratory experiments seek to measure a process with respect to one variable, whilst keeping all other variables constant. In field or in situ experiments, however, an attempt must be made to measure, rather than control, as many as possible of the relevant parameters. In a study of photosynthesis, the two foremost environmental parameters are irradiance and carbon supply (see Chapters 4 and 3 respectively). In the in situ experiments, irradiance has been measured at the experimental sites and such measurements are deemed to give a fairly true representation of the in situ light regime. It has already been suggested that the lack of water movement in experimental bottles may occasionally result in a limitation of carbon supply.

Thus although transporting an essentially laboratory technique into the field does result in certain parameters being reproduced faithfully, like irradiance, the necessary modifications to the technqiue for field use (e.g. lack of agitation) can result in other parameters being poorly reproduced. It is felt important however, that in situ experiments be carried out in order to establish the relevance of laboratory experimental work to the natural situation.

It is important to differentiate between the two types of under-water experiment involved in the present study. These were (a) the "true" in situ experiment in which algae were incubated at their site of collection and normal growth, and (b) a more experimental approach in which algae were transported from their site of collection and normal growth to another depth for incubation. The former type sought to measure the normal,

natural photosynthetic rates of algae in their habitat, the latter to investigate the degree of adaptation of the individual species to their normal growth depths.

In the present study, it has been accepted that of the relevant variables, that which undergoes most change with depth is irradiance, which changes in quantity and, secondarily, quality. In considering irradiance as a limiting factor in photosynthesis, four stages can be recognised, (a) "compensation", below which irradiance is insufficient to promote carbon fixation greater than respiratory loss of carbon, (b) "limitation", where the photosynthetic rate is linearly related to irradiance, (c) "saturation", where a further increase in irradiance produces no corresponding increase in photosynthesis due to some other limiting factor, (d) "inhibition", when irradiance is sufficiently high to induce a decrease in photosynthesis due to destructive or inefficient procesees.

In the present study, frequently only two experimental stations, one "deep", one "shallow", were possible in the course of one experiment, due to the time consuming nature of the diving techniques involved. The results of such experiments have been plotted with photosynthetic rate on the ordinate axis and depth on the abscissa. Classical phytoplankton studies (see review by Yentsch 1963) usually place depth on the vertical or ordinate axis as in the sea, but it is felt that due to the proximity and influence of the shore and sea floor in these experiments on attached macroalgae, the meaning of the results is clearer with depth on the abscissa, implying an increased depth with increased distance from the shore. Also, photosynthesis-irradiance curves to which the photosynthesis-depth curves are analogous, are always plotted with irradiance on the abscissa, following the usual scientific procedure of placing the independent variable on

the abscissa and the dependent variable on the ordinate. In the figures, the mean values of photosynthetic rates calculated for different depths have been joined by straight lines but, as shown in the discussion, this is no way implies that photosynthesis always has a linear relationship to depth, but serves rather to show the relation between the rates at the small number of depths studied, being in this respect probably clearer than, for example, histograms. In this connection also, results are plotted as absolute rates rather than rate relative to, for instance, the rate at normal depth of growth. Absolute rates are essential when considering the productivity of a community, and also when comparing with the data of other workers, although this may not always be valid due to the wide variety of experimental conditions.

Both at Ganzirri and British sites, incubations were generally carried out above any algal canopy or on "open" unshaded parts of the sea floor. This was principally to allow the study of only one independent variable, viz. the reduction of irradiance due to increasing depth. The reduction of irradiance by the canopies has already been considered (Chapter 4) and photosynthesis beneath canopies has been measured in the present chapter. Several of the species studied do not normally live below a canopy in any case, e.g. Porphyra umbilicalis, Laurencia pinnatifida, but are intertidal. In these cases, shallow incubations seek to indicate the photosynthesis occurring when these algae are covered by tides.

In the tables, results have been expressed as described in Chapter 2 and conversion factors have been used to produce, for as many species as possible, values expressed as both $\mu gC cm^{-2} h^{-1}$ and $\mu gC mg^{-1} h^{-1}$ (using a

photosynthetic quotient of 1 as stated in Chapter 2). In each table, only results directly derived from measurements made in the individual experiments carry standard errors; values not carrying standard errors have been derived by conversion either using the factors in Table 2.5 for conversion between oxygen evolved and carbon dioxide fixed, or the SLA of the species in question for conversion between rates expressed per unit weight and per unit area.

Photosynthetic efficiencies have been included where possible, in the tables, and though discussed in detail in Chapter 7, are useful here for comparative purposes. The values represent the ratio of "energy-equivalent of carbon fixed : energy available to the plant as PAR". As described in Chapter 7, this can be derived from the relation

$$\text{Photosynthetic efficiency} = \frac{\mu gC \text{ fixed}}{J \text{ PAR available}} \times 4.1855$$

The value cannot readily be computed from photosynthetic rates expressed solely on a dry weight basis.

2. Experiments conducted at Ganzirri

Environmental parameters

For the majority of these experiments, i.e. those conducted in September, the experimental parameters were:

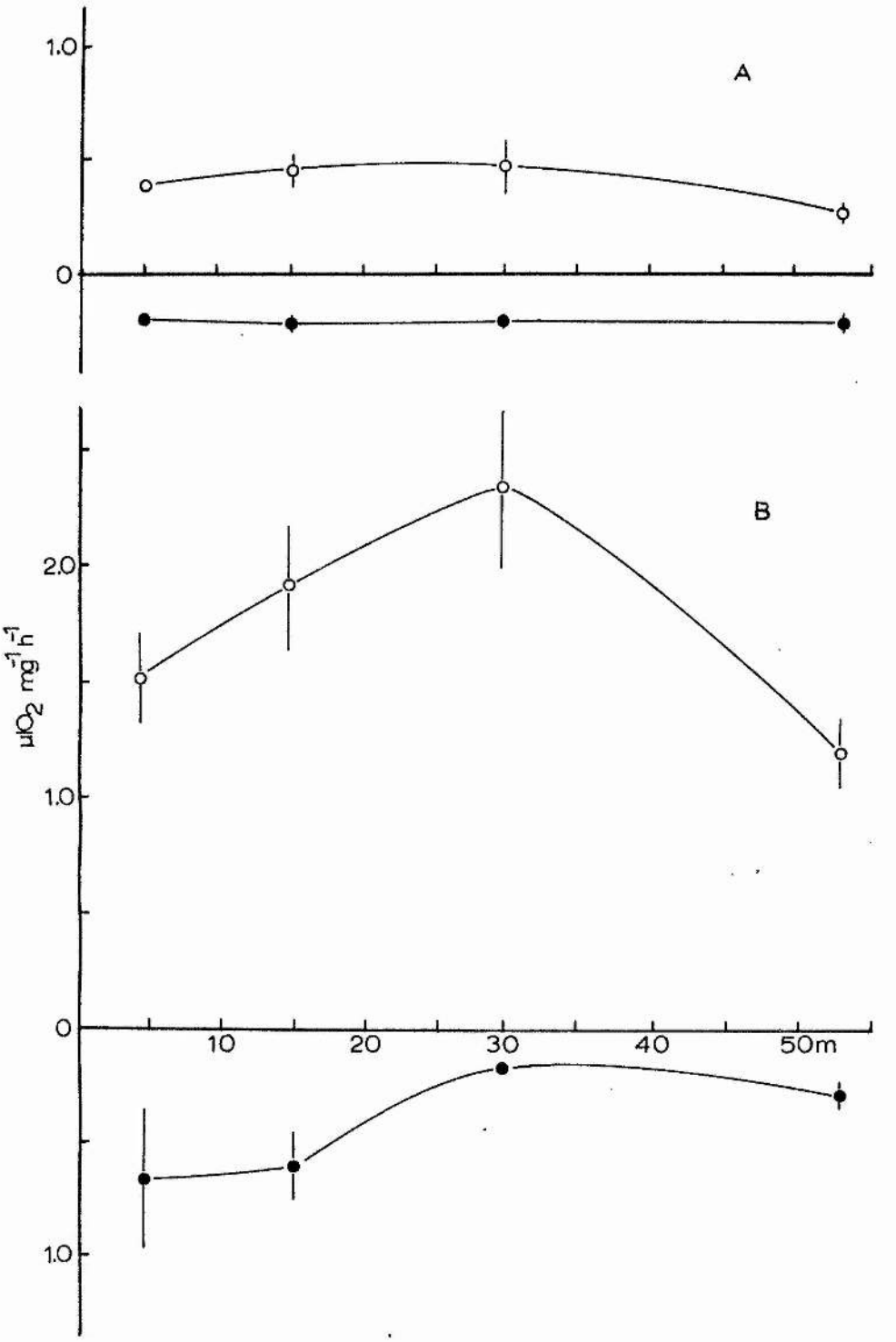


Figure 6.1. Rates of photosynthesis and respiration measured in situ at four depths at Ganzirri in September, using the oxygen method; A, Sphaerococcus; B, Ulva. Incubated for 6-8h at 14.5-24.0°C (see p.172). Open symbols represent photosynthesis, closed symbols dark respiration.

Temperature:	14.5 to 24.0°C (see Chapter 5)
Irradiance:	surface mean 18 mW cm^{-2} PAR; depth 4.5 m, 11 mW cm^{-2} depth 15 m, 4.0 mW cm^{-2}; depth 30 m, 1.2 mW cm^{-2}; depth 53 m, 0.20 mW cm^{-2}.
Duration:	6 - 8 h, generally between 10.00 h and 18.00 h BST

In experiments carried out in April the most significant difference was in temperature, which was close to 14.5°C throughout the depth profile (see Chapter 5). Irradiance was not measured, but continuous sunlight prevailed during the experiments and according to de Jong (1973), the irradiance in this region is very similar in April and September.

a. Photosynthesis of algae incubated at their normal depth of growth (oxygen and ^{14}C methods)

The results of a series of thirteen experiments (oxygen method) conducted in situ on Sphaerococcus and Ulva are shown in Figure 6.1. In both species, the photosynthetic rates were highest in the middle of the depth range. In Ulva the rate at 30 m was significantly higher than at 5 m or 53 m. The photosynthetic rates of specimens of Sphaerococcus growing and incubated at 53 m were significantly lower than for those at 15 or 30 m. When considered on a dry weight basis, the rates for Ulva were about four times those for Sphaerococcus.

Table 6.1 shows rates of photosynthesis of Laurencia, Gracilaria, Pterocladia, Vidalia, Peyssonelia and Pseudolithophyllum, in each case measured at one depth only. Mean values for Sphaerococcus and Ulva (from Figure 6.1) are included for comparison.

Table 6.1 Net photosynthesis of algae incubated at their normal depth of growth at Ganzirri in September, temperature 14.5-24.0°C (oxygen method)

Species	Depth m	n	Photosynthesis $\mu l O_2\ mg h^{-1}$	$\mu gC mg h^{-1}$	$\mu gC cm^{-2} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$ PAR	Efficiency %
Laurencia	4.5	18	1.87±0.11	1.00			
"	15	6	1.47±0.01	0.78			
Gracilaria	4.5	4	0.93±0.08	0.50			
Pterocladia	4.5	2	0.89±0.15	0.48			
Vidalia	15	2	1.70±0.14	0.38			
Sphaerococcus	4.5	2	0.33±0.06	0.18			
"	15	10	0.46±0.05	0.25			
"	30	8	0.48±0.04	0.26			
"	53	8	0.27±0.04	0.15			
Peyssonelia	53	2	0.08	0.04	0.60±0.09	0.65	3.86
" [a]	53	2	0.24	0.13			
Pseudolitho-phyllum	53	2	0.02	0.01	0.81±0.19	0.65	5.22
" [a]	53	2	0.18	0.10			
Ulva	4.5	12	1.52±0.18	0.82	1.07[b] (2.54)[c]	32.4	0.14
"	15	4	1.92±0.26	1.03	0.93 (2.22)		
	30	4	2.38±0.35	1.28	1.15 (2.75)		
	53	8	1.17±0.14	0.63	0.57 (1.36)	0.65	3.67

[a] Based on decalcified dry weight

[b] Calculated using extracted SLA values from Table 5.6

[c] Calculated using unextracted SLA values from Table 5.6

On a dry weight basis, maximum rates were attained by the green alga Ulva growing at 30 m and the red Laurencia at 4.5 m. Sphaerococcus had a uniformly low rate at all depths compared with the shallow species, but comparable to rates recorded for the calcified deep water species Peyssonelia and Pseudolithophyllum, considered on a decalcified, or organic matter basis. On a dry weight basis, the rates of these latter two species were lower by a factor of about ten than the results for noncalcified species.

Considering the results for Ulva, Peyssonelia and Pseudolithophyllum calculated on an area basis the rates for the two rhodophyte species were closely comparable with those for Ulva. When incubated at 53 m the efficiencies computed for these species using extrapolated values for irradiance were of the order of 5%.

In all experiments, a net evolution of oxygen was recorded and thus there was no indication that algae were below compensation point during the relatively short duration of the experiments.

The results of one experiment conducted in September and two in April, using the ^{14}C method are shown in Table 6.2.

Table 6.2 Photosynthesis of algae incubated at their normal depth of growth at Ganzirri, temperature 14-24°C (^{14}C method)

Species	Month	Depth m	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Photosynthesis $\mu gC mg^{-1}$ [a] h^{-1}	Irradiance [b] J $cm^{-2} h^{-1}$ PAR	Efficiency %
Sphaerococcus	Sept	53		0.61±0.08	0.65	
Gracilaria	Sept	4.5		12.38±5.86	32.40	
Ulva	Sept	4.5	3.63±0.13 [c]	2.80±0.00	32.40	0.13
"	Sept	53	3.87±0.96 [c]	4.06±0.09	0.65	24.92
"	April	4.5	10.90±0.00 [c]	22.10±0.00	32.40	1.41
Peyssonelia	April	60	3.18	0.26	0.65	20.48
Pseudolithophyllum	April	60	4.72	0.11	0.65	30.39
Porphyra	April	4.5	17.91±3.29	57.30±10.53	32.40	2.31

a Extracted dry weight

b Assuming a mean irradiance of 18mW cm^{-2} PAR (65 J $cm^{-2} h^{-1}$) at surface

c Alcohol extracted SLA values were 0.77, 1.05 and 2.03 $cm^2 mg^{-1}$ respectively

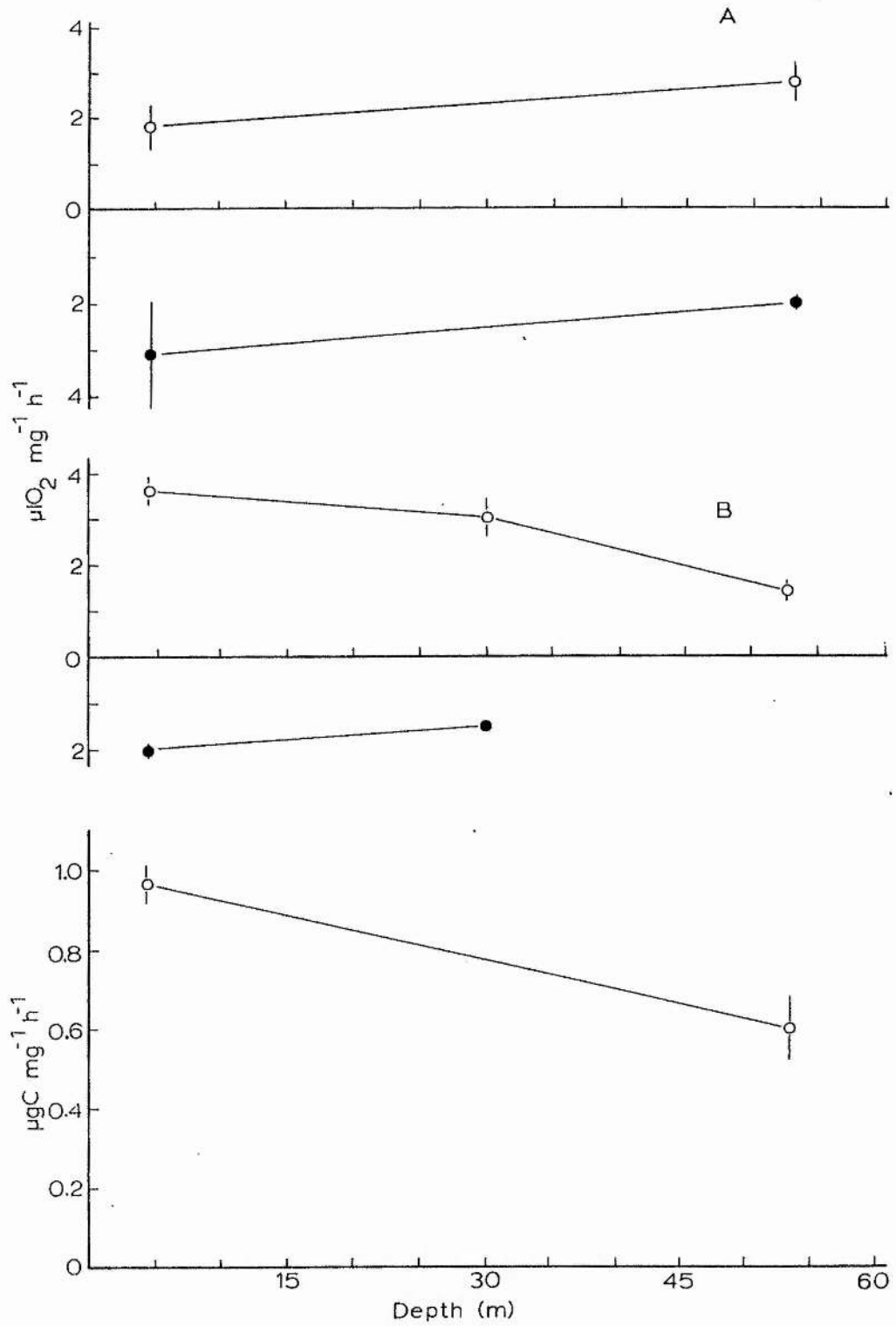


Figure 6.2. (upper). Rates of photosynthesis and respiration of Sphaerococcus in situ and after transfer to different depths, at Ganzirri in September using Oxygen method, A, tissue from 53m; B, tissue from 4.5m. (Conditions and symbols as for Figure 6.1).

Figure 6.3. (lower). Rates of photosynthesis of Sphaerococcus (source 53m) measured in situ and after transfer to 4.5m at Ganzirri in September, using ^{14}C method (Conditions as for Figure 6.1.).

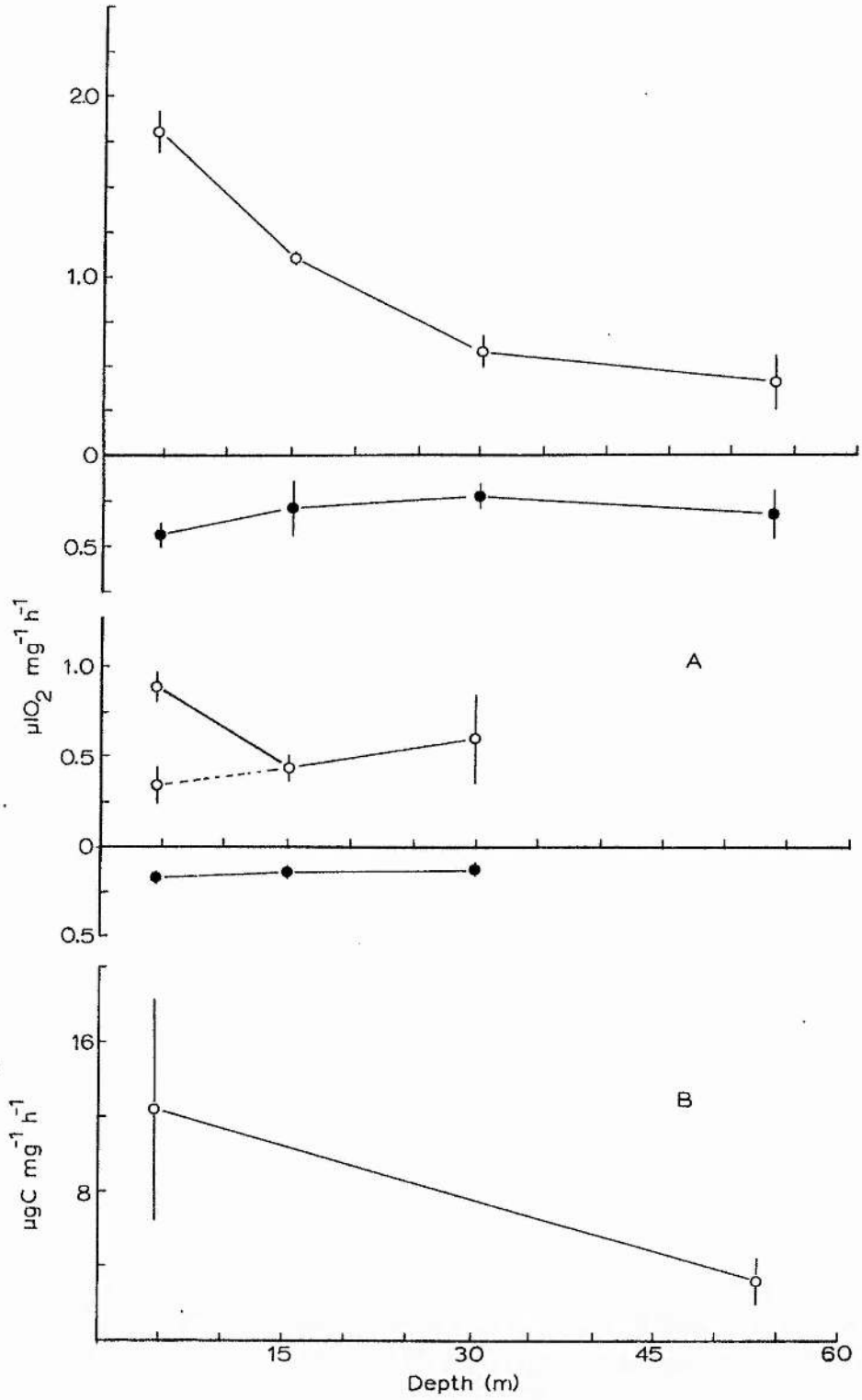


Figure 6.4. (upper). Rates of photosynthesis and respiration of Laurencia (source 4.5m) measured in situ and after transfer to greater depths at Ganzirri in September, using oxygen method (Conditions and symbols as for Figure 6.1.).

Figure 6.5. (lower). Rates of photosynthesis of Gracilaria (source 4.5m) measured in situ and after transfer to greater depths at Ganzirri in September; A, using oxygen method, in the "open" (solid lines), in canopy shade (broken line); B, using ^{14}C method. (Conditions and symbols as for Figure 6.1.).

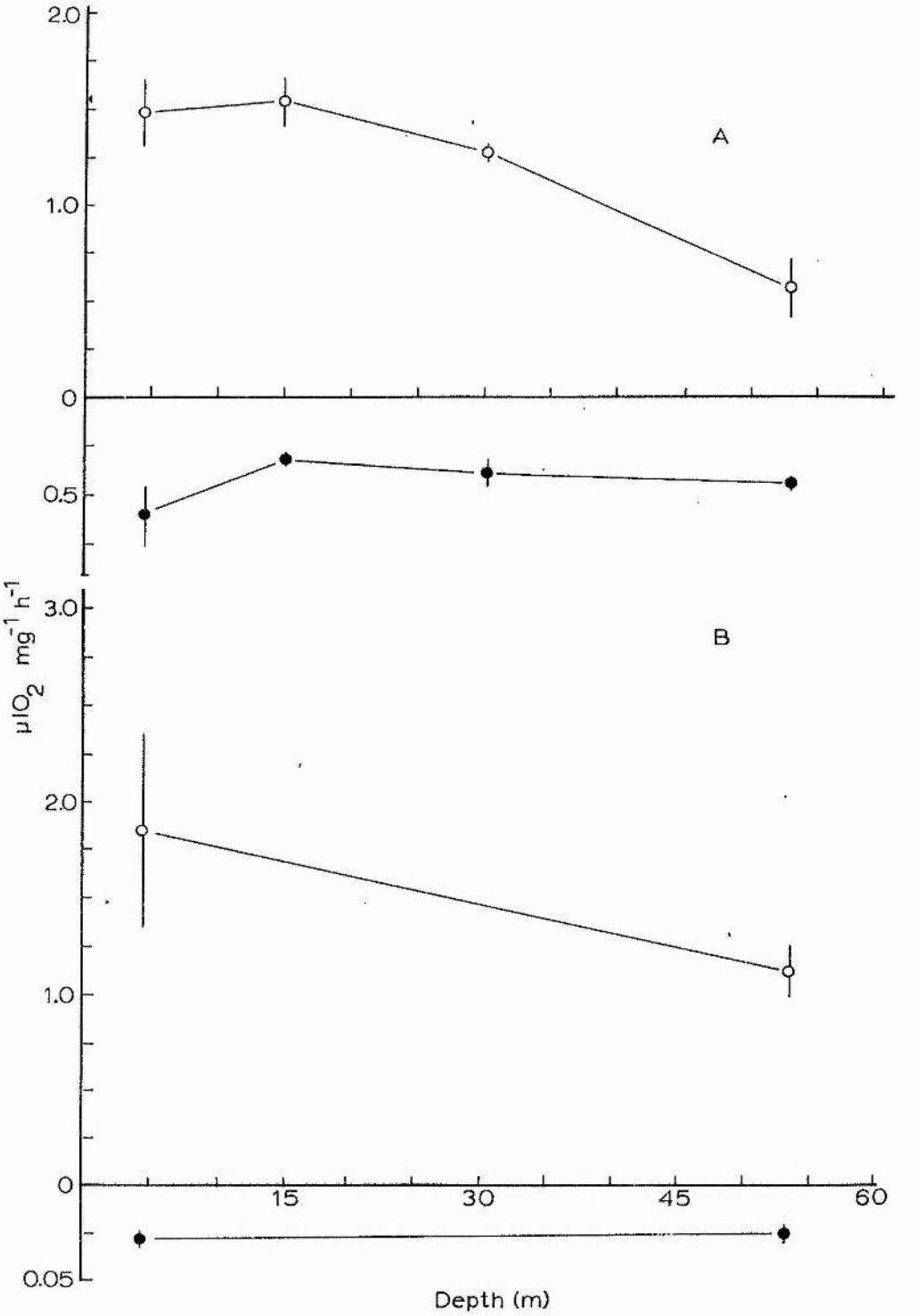


Figure 6.6. Rates of photosynthesis and respiration of Ulva at Ganzirri in September, measured in situ and after transfer to different depths using the oxygen method; A, tissue from 4.5m; B, from 53m. (Conditions and symbols as for Figure 6.1.).

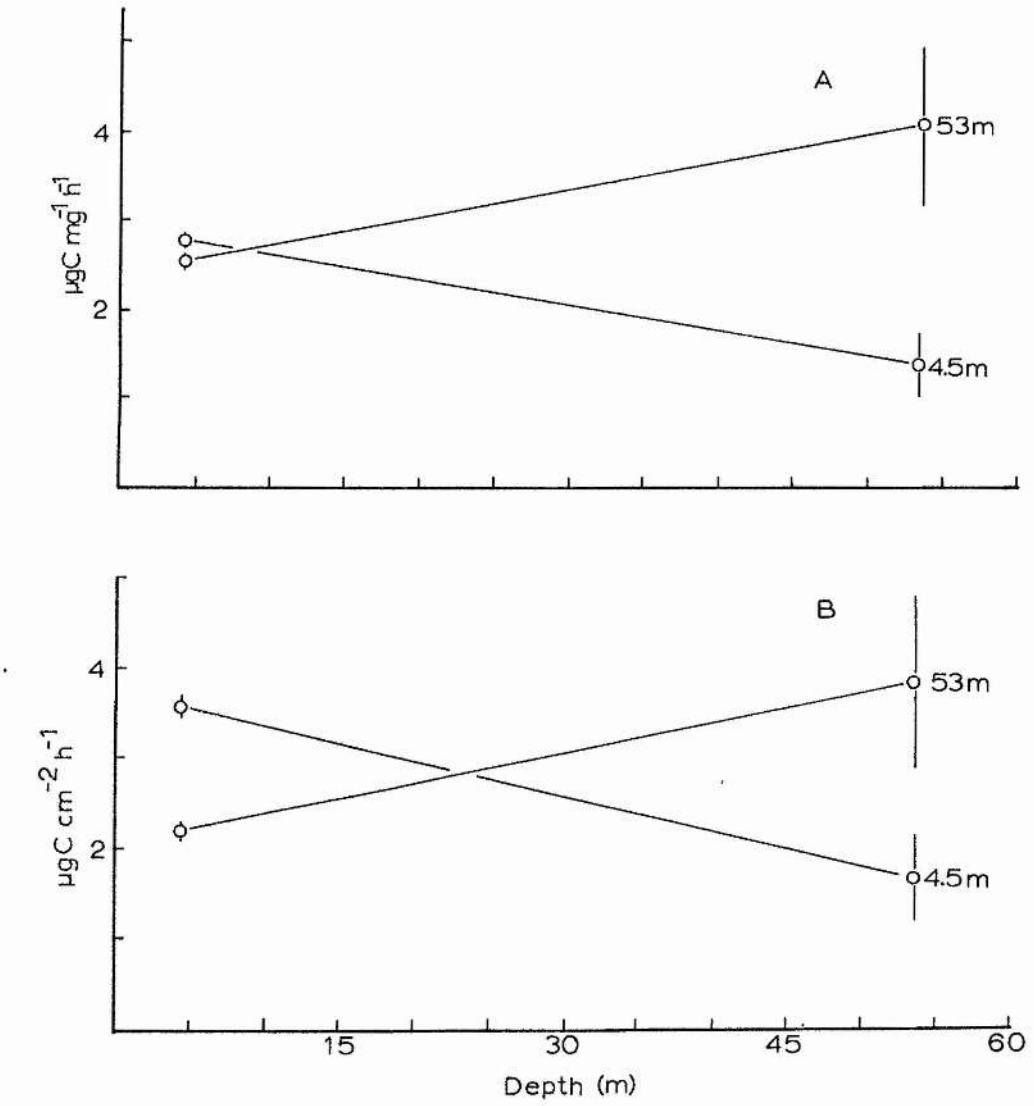


Figure 6.7. Rates of photosynthesis of Ulva (sources 4.5m and 53m) measured in situ and after transfer, at Ganzirri in September using ^{14}C method; A, expressed on dry weight basis; B, expressed on area basis. (Conditions and symbols as for Figure 6.1.).

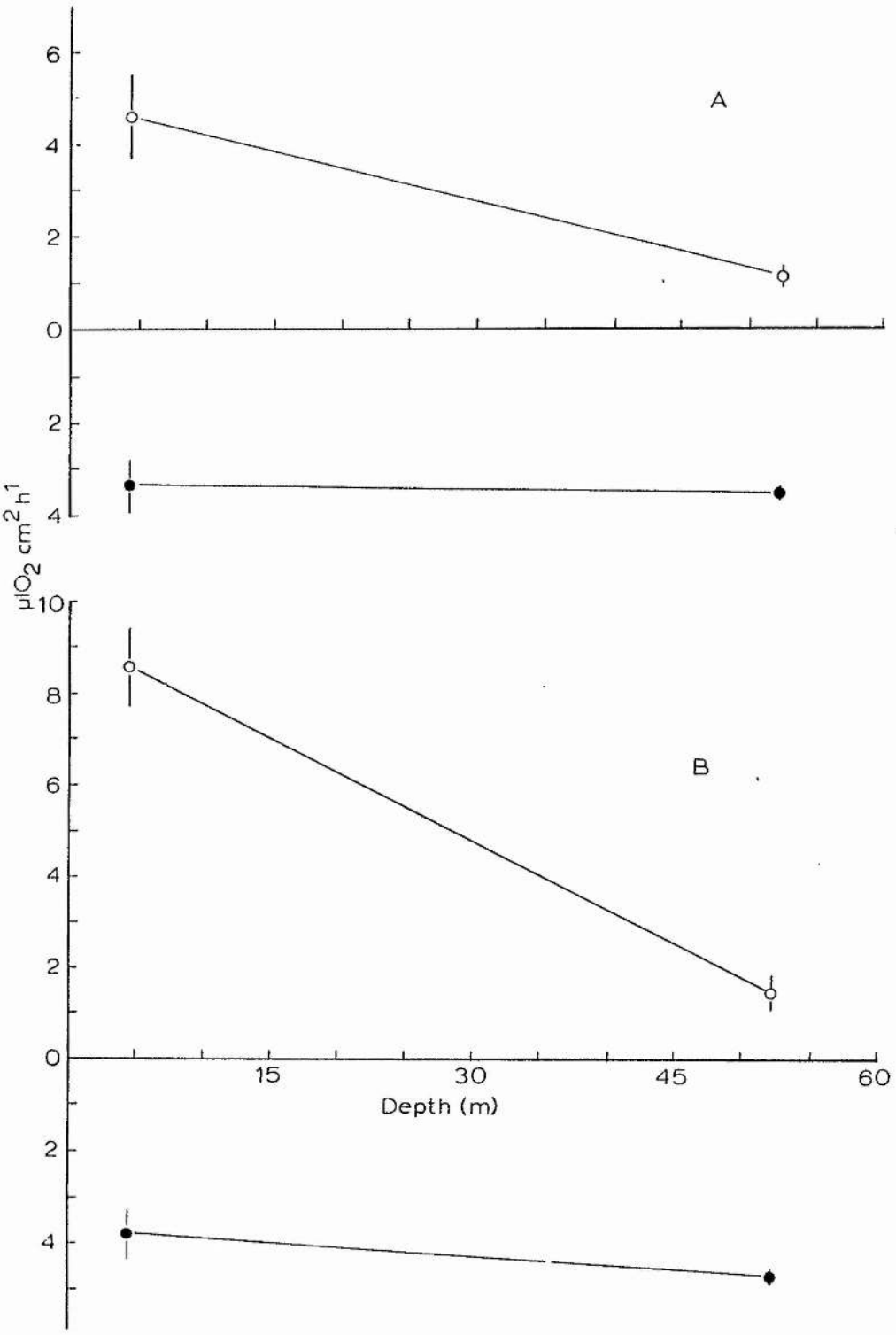


Figure 6.8. Rates of photosynthesis and respiration measured in situ at 53m and after transfer to 4.5m at Ganzirri in September using oxygen method; A, Peyssonelia B, Pseudolithophyllum. (Conditions and symbols as for Figure 6.1.).

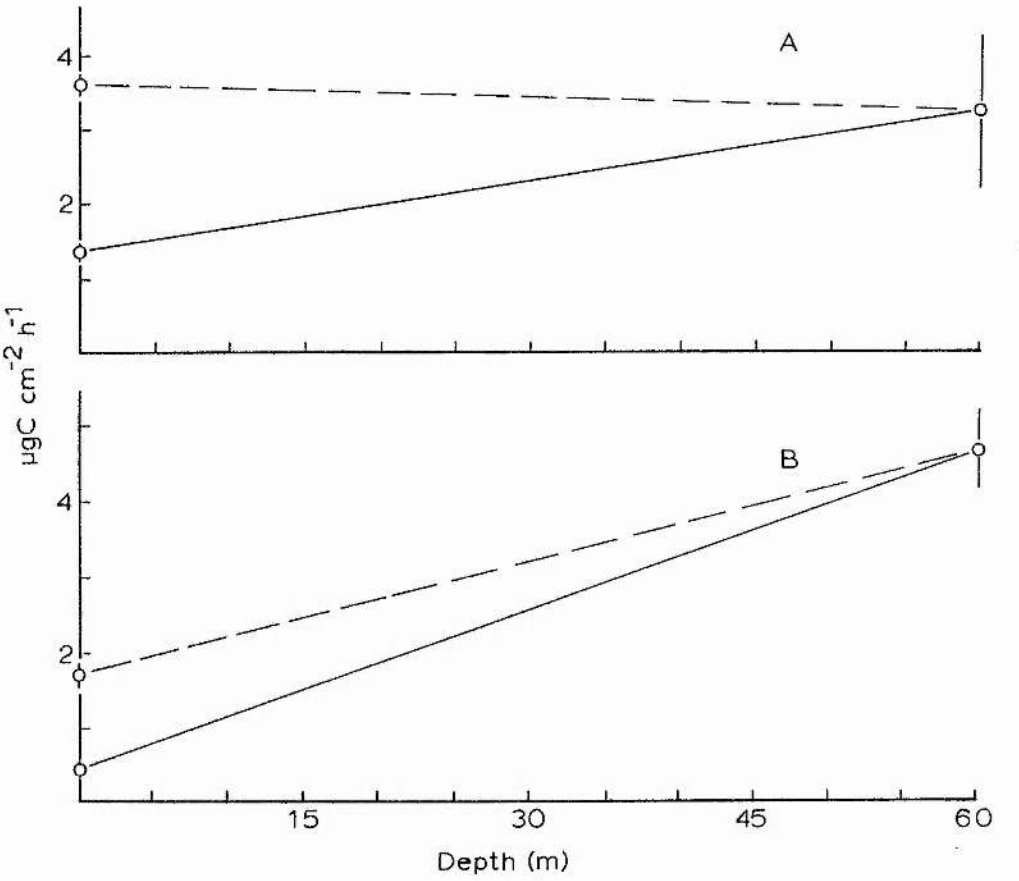


Figure 6.9. Rates of photosynthesis measured in situ at 60m, and after transfer to 0m at Ganzirri in April using ^{14}C method; A, Peyssonelia; B, Pseudolithophyllum. Incubation times at surface, 1.5h (broken lines) and 5h (solid lines); at 60m, 5h only.

Quantitatively the rates are much higher than those measured by the oxygen method (Table 6.1), as discussed in Chapter 2. The rates for Ulva at 4.5 and 53 m in September were not significantly different but both were significantly higher than the rate for Sphaerococcus at 53 m; these relationships are in general agreement with the results of the oxygen method experiments. On an area basis, the rates for Peyssonelia and Pseudolithophyllum in April were similar to those for Ulva in September. The results for Ulva and Porphyra in April were very high and will be discussed later. Due to the higher rates measured by the ^{14}C experiments compared with the oxygen method, the computed efficiences shown in Table 6.2 for algae incubated at 53 m and 60 m were extremely high, being in the region of 20 - 30%.

b. Photosynthesis of algae when transferred to various depths (oxygen and ^{14}C methods)

Figures 6.2 - 9 present the results of twenty six experiments conducted underwater in a study of six algal species. In general the results showed that when algae were transferred from a shallow (4.5 m) to deeper sites, photosynthetic rates decreased. In Laurencia (Figure 6.4) this decrease was fastest at the shallow depths from 4.5 - 15 m. In Ulva (Figure 6.6A) rates were fairly uniform from 4.5 - 33 m, dropping to a significantly lower rate at 53 m. Gracilaria (Figure 6.5A) gave variable results but showed a general decrease in photosynthetic rates with depth when incubated in the "open". Specimens incubated in their natural habitat in the shade beneath the Cystoseira canopy had the lowest rates. Shallow-water specimens of the deep-water species Sphaerococcus showed a gentle slope decreasing to 53 m (Figure 6.2B). The results of ^{14}C experiments on Ulva

(Figure 6.7A and B) and Gracilaria (Figure 6.5B) both from 4.5 m, also showed a decrease in photosynthetic rate with depth.

When transferred from the deepest sites to the shallows, deep-growing specimens mostly showed increased rates, viz. Ulva (Figure 6.6B), Peyssonelia (Figure 6.8A) and Pseudolithophyllum (Figure 6.8B) in experiments using the oxygen method, and Sphaerococcus, (Figure 6.3), using the ^{14}C method. Ulva (Figure 6.7A and B) with the ^{14}C method, however, showed a reduction, or inhibition, of photosynthetic rate at 4.5 m and Sphaerococcus, using the oxygen method (Figure 6.2A) also showed a reduction in mean rate at 4.5 m although this was not statistically significant. It should be noted that, due to the different SLA values for Ulva from 4.5 m and 53 m, the relationship between 4.5 m and 53 m material is different when expressed on a dry weight or on an area basis (Figure 6.7A and B).

A striking difference is seen between the response of the deep algae (source 53 m and 60 m), Peyssonelia and Pseudolithophyllum, to shallow incubation in September (Figures 6.8A and B) and in April (Figures 6.9A and B). In September the rate of photosynthesis of both species was higher at 4.5 m (incubation time, 6.75 h) than at the natural depth of 53 m (Figure 6.8). In April however, those incubated at 0 m for 5 h showed a great depression or inhibition of photosynthetic rate compared with those at 60 m incubated for the same length of time. When specimens were incubated at 0 m for only 1.5 h, rates were significantly higher than the 5 h rates, and in the case of Peyssonelia the mean value slightly exceeded that at 60 m.

c. Photosynthetic rates measured before and after noon (oxygen method)

Making use of the unusual occurrence of three slack water periods during one day at dawn, noon and dusk (Chapter 5) one experiment was conducted

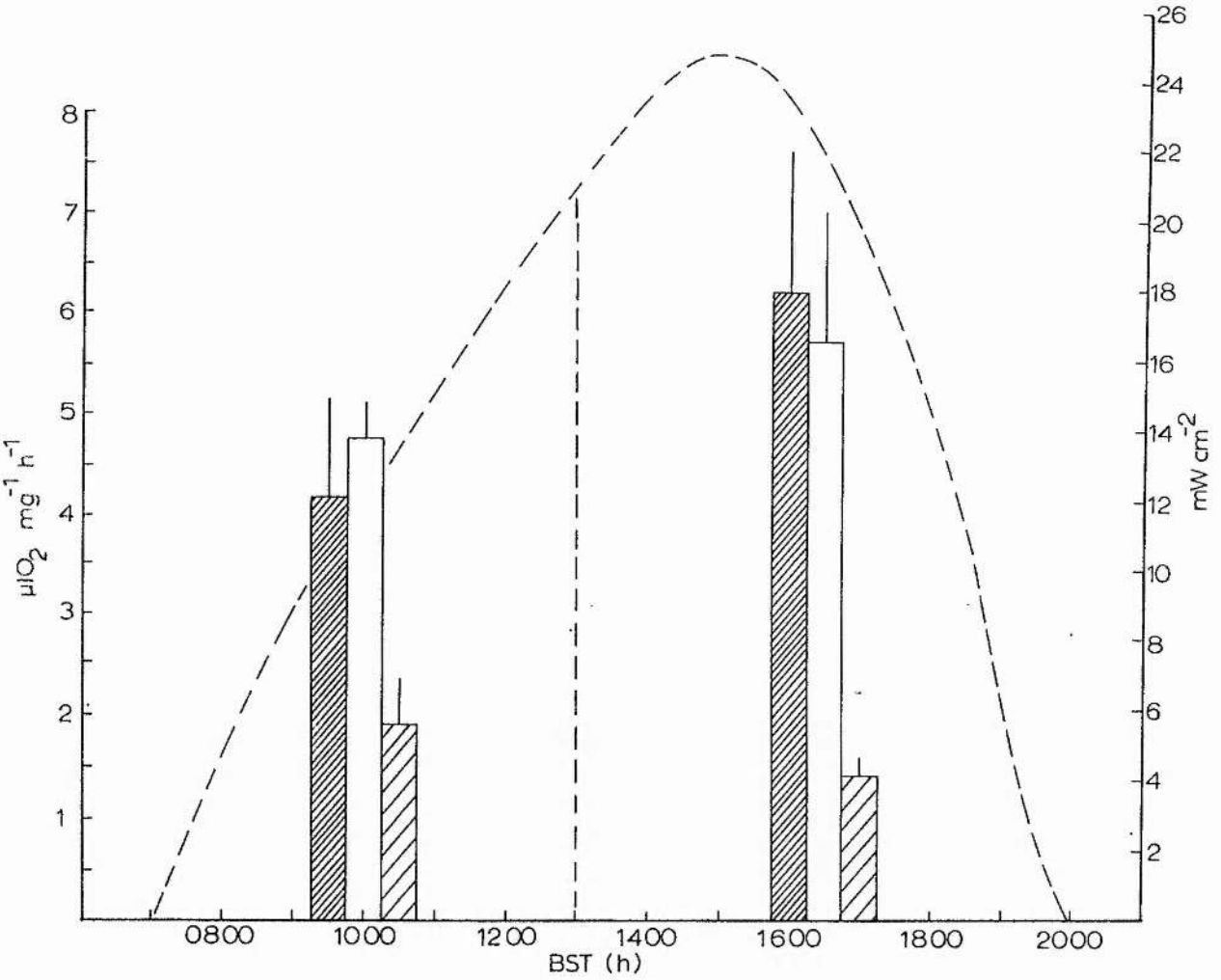


Figure 6.10. Photosynthetic rates measured before and after noon at 15m at Ganzirri in September, using the oxygen method (with solar irradiance curve superimposed); Laurencia, source 15m (closely hatched columns); Laurencia, source 4.5m (clear columns; Sphaerococcus, source 15m (hatched columns).

involving separate morning (0730 - 1300 h BST) and afternoon (1300 - 1900 h BST) incubation periods, in an attempt to determine if there was any marked daily periodicity of photosynthetic rate. The experiment was conducted at a depth of 15 m using Sphaerococcus (source 15 m) and Laurencia (sources 15 and 4.5 m) and mean hourly rates for morning and afternoon are shown in Figure 6.10. The daily irradiance curve is superimposed in the figure, showing that total irradiance was substantially greater (about twice) in the afternoon than in the morning period, which was also 0.5 h shorter. Both Laurencia samples showed slightly higher rates in the afternoon but Sphaerococcus showed a slight decrease. The results give reason to conclude that there is no appreciable photosynthetic periodicity attributable to time of day per se in these algae.

d. Seasonal variation in photosynthetic rate

Certain experiments on Sphaerococcus and Ulva in both September and April were conducted under conditions closely similar in respect of source material, site of incubation and irradiance, and so permit a comparison of photosynthetic activity on a seasonal basis. The results of oxygen method experiments presented in Table 6.3, and ^{14}C experiments on Ulva in Table 6.2 show that photosynthetic rate was higher in both species in April (this being accompanied by a higher respiration rate - Chapter 8). The most striking case is the value of 22.1 $\mu gC mg^{-1} h^{-1}$ computed for Ulva using the ^{14}C method in April which is due to the combined effects of high rate per unit area (i.e. 10.9 $\mu gC cm^{-2} h^{-1}$) and extremely high SLA (2.03 $cm^2 mg^{-1}$).

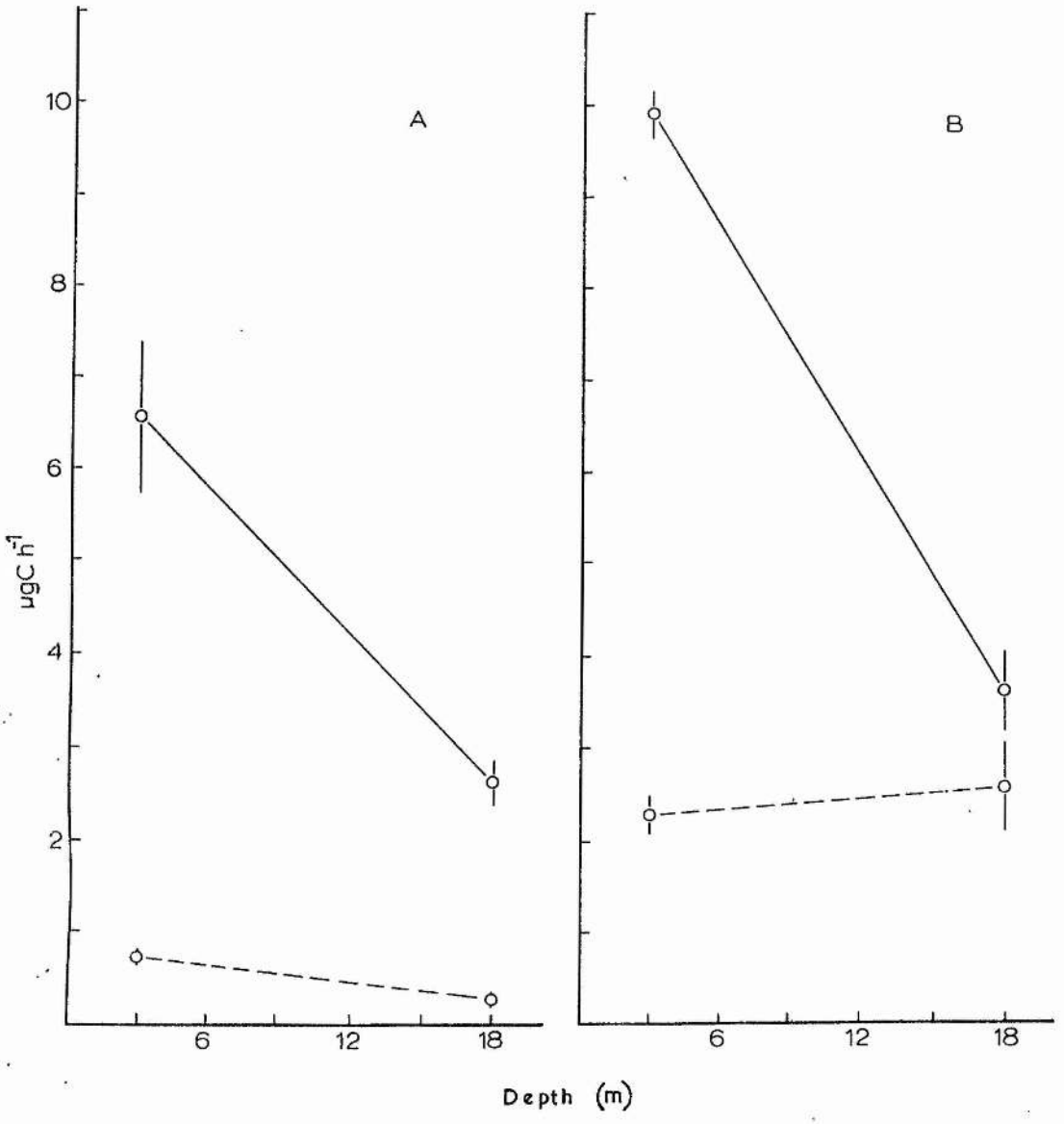


Figure 6.11. Rates of photosynthesis measured in situ at two depths at Puffin Island; A, Dilsea; B, Ulva. Rates expressed on area basis, µgC cm^{-2} h^{-1} (solid lines) and dry weight basis µgC mg^{-1} h^{-1} (broken lines). Conducted in July; ^{14}C method; 13°C; 14h.

Table 6.3 Photosynthesis of Sphaerococcus and Ulva in September and April (oxygen method)

Species	Month	Depth m	n	Photosynthesis $\mu lO_2\ mg^{-1}\ h^{-1}$	$\mu gC\ mg^{-1}\ h^{-1}$	$\mu gC\ cm^{-2}\ h^{-1}$
Sphaerococcus (source 53 m)	Sept	4.5	5	0.18 ± 0.05	0.10	
Sphaerococcus (source 53 m)	April	4.5	6	2.11 ± 0.40	1.14	
Ulva (source 4.5 m)	Sept	4.5	6	1.52 ± 0.18	0.82	1.07
Ulva (source 4.5 m)	April	4.5	4	3.72 ± 0.64	2.00	0.99

3. Experiments conducted in Britain

a. Puffin Island (^{14}C method)

The results of three experiments carried out on Dilsea and Ulva at their natural depths of 3 m and 18 m are shown in Figure 6.11A and B. Expressed on an area basis, the results for both species indicated that carbon fixation by the plants growing at 18 m was less than half that attained by plants growing normally growing at 3 m. In quantitative terms, the rates for the two species at any one depth were of the same order of magnitude. When expressed per unit unextracted dry weight however, the rates for Dilsea were up to ten times lower than those for Ulva due to the very low SLA values (0.099 - 0.113 cm^2mg^{-1}) for the former species compared with the latter (0.267 - 0.880 cm^2mg^{-1}). Similarly, due to the differential between the SLA

of Ulva from 3 m and from 18 m, the mean photosynthetic rates expressed per gram unextracted dry weight were not significantly different at the two depths, implying that the relative growth rates may in fact be similar.

The results of a further eight experiments conducted on several algal species at their natural depth of growth at Puffin Island are presented in Table 6.4.

Table 6.4 Photosynthesis (^{14}C method) measured at Puffin Island in July, temperature 13°C. Natural depths.

Species	Depth m	n	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Photosynthesis $\mu gC mg^{-1} h^{-1}$	SLA $cm^2 mg^{-1}$	Irradiance $Jcm^{-2} h^{-1}$ PAR	Efficiency %
Porphyra umbilicalis	0	2	6.81±0.09	1.31	0.192	70.8	0.40
Porphyra umbilicalis	3	4	6.34±1.04	2.24	0.353	76.4	0.35
Porphyra leucosticta	3	2	6.47±0.42	3.84	0.594	70.6	0.39
Rhodymenia (red)	3	2	11.95±1.45	2.04	0.171	69.7	0.72
" (yellow)	3	2	11.70±5.82	1.99	0.170	69.7	0.70
Dilsea	3	4	6.57±1.77	0.66	0.100	57.0	0.48
Enteromorpha	3	2	15.15±1.04	7.05	0.465	70.1	0.91
Ulva	3	2	9.89±0.26	2.29	0.232	43.6	0.95
Laurencia (red)	3	2		5.72±0.48[a]			
" (yellow)	3	2		4.94±0.19[a]			
Dilsea	18	2	2.61±0.25	0.26	0.100	2.88	3.79
Delesseria	18	4	3.05±0.57	0.91	0.298	3.78	3.37
Phycodrys	18	4	1.78±0.26	0.69	0.387	3.02	2.47
Polyneura	18	2	0.59±0.10	0.39	0.669	3.15	0.78
Nitophyllum	18	2	2.39±0.03	0.85	0.357	4.24	2.36
Kallymenia	18	2	1.28±0.01	0.50	0.391	3.15	1.70
Ulva	18		3.63±0.43	2.60	0.716	4.24	3.58

a Alcohol extracted dry weight basis

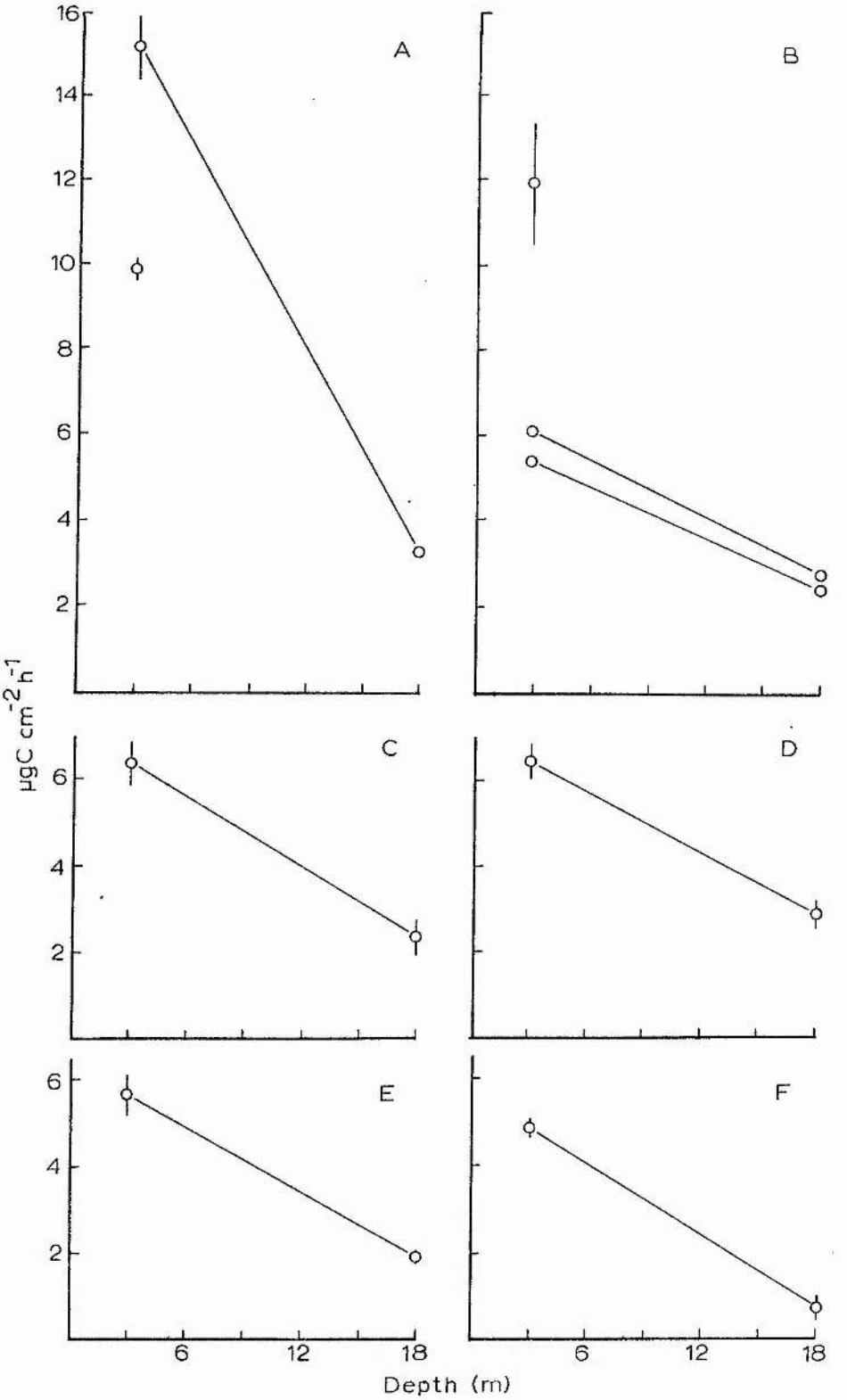


Figure 6.12. Rates of photosynthesis of shallow algae at Puffin Island measured in situ at 3m, and after transfer to 18m; A, Enteromorpha; and Ulva (single point); B, Rhodymenia (single point) and Dilsea, red tissue (upper line) and yellow tissue Conducted in July; ^{14}C method; 13°C; ~4h.

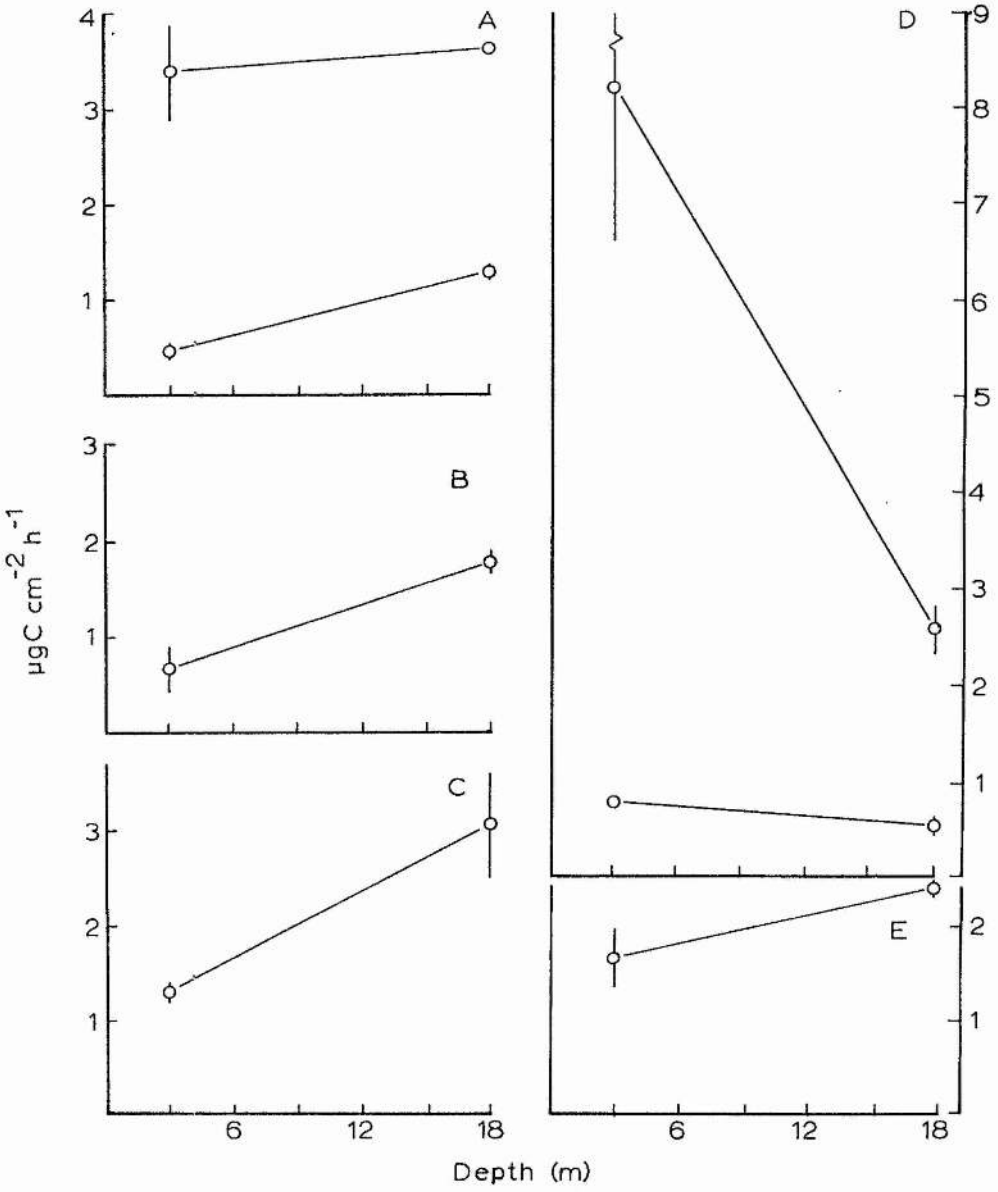


Figure 6.13. Rates of photosynthesis of deep algae at Puffin Island measured in situ at 18m, and after transfer to 3m; A, Ulva (upper curve) and Kallymenia (lower curve); B, Phycodrys; C, Delesseria; D, Dilsea (upper curve) and Polyneura (lower curve); E, Nitophyllum Conducted in July; ^{14}C method; 13°C; ∿4h

Carbon fixation rates were invariably higher for algae of the littoral and upper sublittoral zones, where of course irradiances were high. The highest rate was attained by the green alga Enteromorpha (15 $\mu gCcm^{-2} h^{-1}$) followed by the red Rhodymenia (12 $\mu gCcm^{-2} h^{-1}$). Of the deep sublittoral algae, the highest rate was again attained by a green alga, Ulva (3.5 $\mu gCcm^{-2} h^{-1}$) followed by the typical deep-water and shade-loving red alga Delesseria (3 $\mu gCcm^{-2} h^{-1}$). On an unextracted dry weight basis, Enteromorpha had the highest fixation rate of 7.05 $\mu gCmg^{-1} h^{-1}$, and the massive Dilsea, incubated at both depth sites, had the lowest, from 0.657 to 0.261 $\mu gCmg^{-1} h^{-1}$.

In Rhodymenia and Laurencia, yellow "bleached" specimens carried out carbon fixation at a rate equivalent to or only slightly less than their red counterparts. In this connection, considering the two Porphyra species studied, P.umbilicalis, collected from the upper littoral, a "sun"habitat, had a pale yellow thallus and had a lower mean photosynthetic rate, although not significantly lower, than the rose-red P.leucosticta collected in the upper sublittoral growing on the stipes of L.hyperborea.

The results of experiments conducted underwater concurrently with those described above are presented in Figures 6.12 and 13 showing carbon fixation rates of algae measured at their normal depth of growth and also transferred to one other, deeper or shallower depth. In a manner similar to the situation at Ganzirri, the rates attained by littoral or upper sublittoral species, when transferred to 18 m, were greatly reduced compared with their rates at 3 m (Figure 6.12A, B, C, D, E and F). When transferred to 3m however, the plants from 18 m showed varied responses. Dilsea, and to a lesser extent Polyneura (both Figure 6.13D), showed an increase in fixation rate when transferred to the higher irradiance at 3 m. The green alga Ulva however, and the red algae Kallymenia, Phycodrys, Delesseria and Nitophyllum, all showed some degree of depression or inhibition of photosynthesis

when transferred to 3 m. The highest efficiences attained were 3.79% by Dilsea and 3.58% by Ulva, both at 18 m.

An experiment was conducted to assess the effect of the shade created by the canopy of Laminaria hyperborea (with some Saccorhiza polyschides) on the measured rate of photosynthesis in Dilsea and Ulva. Specimens of the two species were collected at 4 m depth and incubated above the canopy on the buoyant platform 2.7 m below the surface, and on the rock at the bases of the L.hyperborea stipes at 4.1 m. The results, presented in Table 6.5, show that carbon fixation in both species was dramatically reduced, and in the case of one replicate of Ulva, was as low as the dark carbon fixation control. The irradiance measured below the canopy was extremely low, however, and the efficiency of Dilsea was correspondingly high.

Table 6.5 Photosynthesis of Dilsea and Ulva (source 4 m) incubated above and below the L.hyperborea canopy (^{14}C method)

Species	Depth m	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Photosynthesis $\mu gC mg^{-1} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$ PAR	Efficiency %
Dilsea (above)	2.7	7.40±0.18	0.74	46.00	0.67
" (below)	4.1	1.67±0.32	0.17	0.90	7.77
Ulva (above)	2.7	9.89±0.26	2.47	46.00	0.90
" (below)	4.1	0.15±0.15	0.04	0.90	0.70

b. Dunstaffnage (^{14}C method)

Table 6.6 presents carbon fixation rates attained by various algae incubated at their sites of growth at depths of 0 m, 6 m, and 12 m. (Delesseria was collected slightly shallower, at 6 m as described in Chapter 5, and so represents strictly speaking a "transfer" experiment.)

Table 6.6 Photosynthesis (^{14}C method) measured at Dunstaffnage in August, temperature 13°C.

Species	Depth m	n	$\mu gC cm^{-2} h^{-1}$	$\mu gC mg^{-1} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$	Efficiency %
Polysiphonia (red)	0	2	-	5.72±0.47	19.5	-
Polysiphonia (yellow)	0	2	-	3.51±0.24	19.5	-
Dilsea (red)	6	2	2.65±0.54	0.27	-	-
" (red)	12	4	3.35±0.20	0.34	2.25	6.23
" (yellow)	12	2	3.09±0.24	0.31	2.25	5.74
Delesseria (red)	12	2	3.77±0.90	0.69	7.19	2.19
Delesseria (yellow)	12	2	2.20±0.07	0.36	7.19	1.28
Phycodrys	12	3	2.52±0.19	1.12	2.25	1.12
Ulva	12	4	3.55±0.20	1.14	5.48	2.46

At 12 m, rates were closely similar for Delesseria, Dilsea, Phycodrys and Ulva, to those attained by these species at 18 m at Puffin Island. The highest rate attained sublittorally was 3.77 $\mu gC cm^{-2} h^{-1}$, by red specimens of Delesseria. Dilsea, collected and incubated at 6 m in the brown peaty water at Rudha na Lain (see Chapter 5), had a lower carbon fixation rate than Dilsea

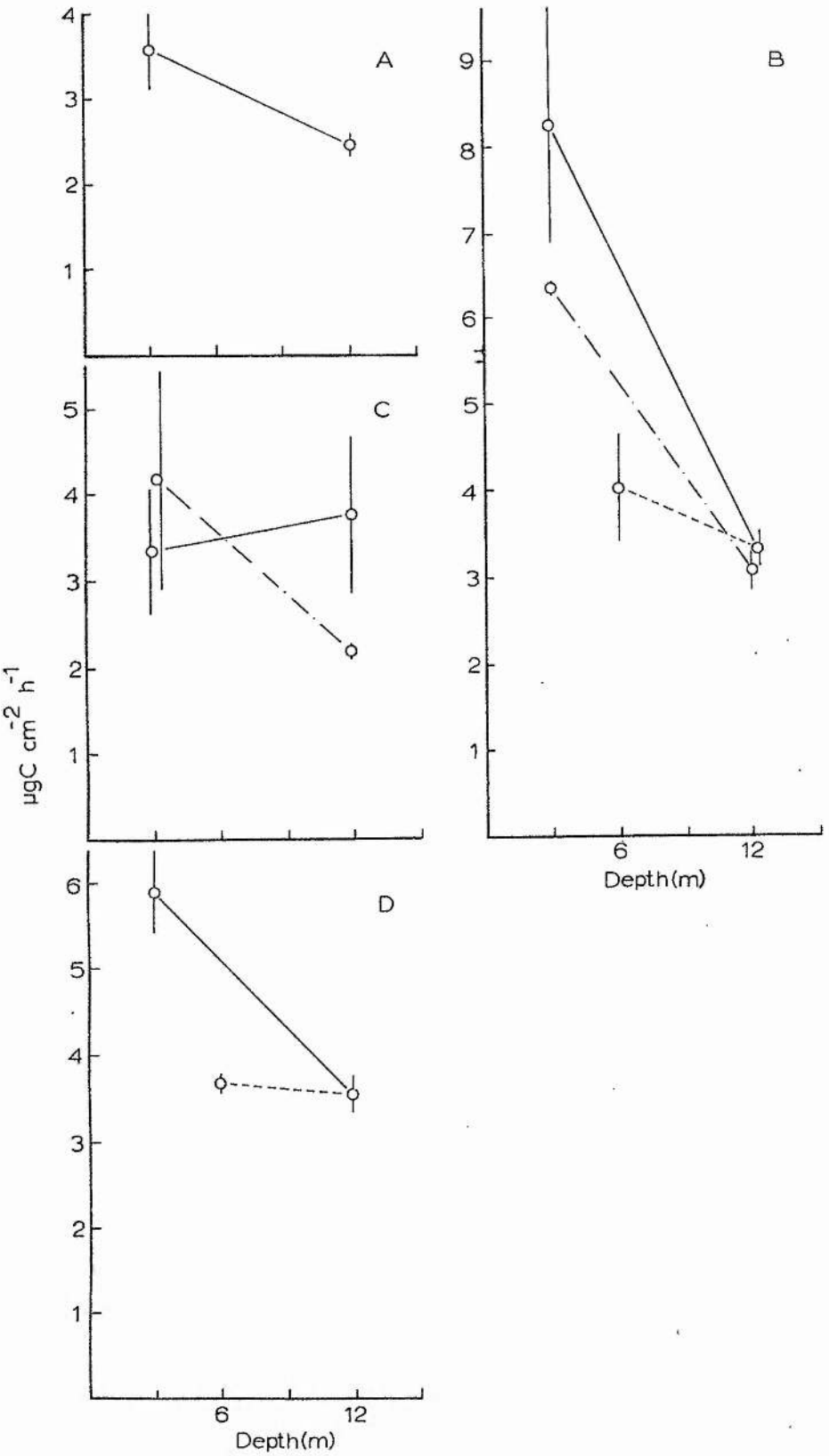


Figure 6.14. Rates of photosynthesis of deep algae at Dunstaffnage measured in situ at 12m and after transfer to 3m; A, Phycodrys; B, Dilsea, red tissue (solid line), yellow tissue (dot-dashed line) and transferred to 6m at Rubha na Lain (broken line); C, Delesseria red tissue (solid line) and green tissue (dot-dashed line); D, Ulva; also transferred to 6m at Rubha na Lain. Conducted in August; ^{14}C method; 13°C; ∿4h.

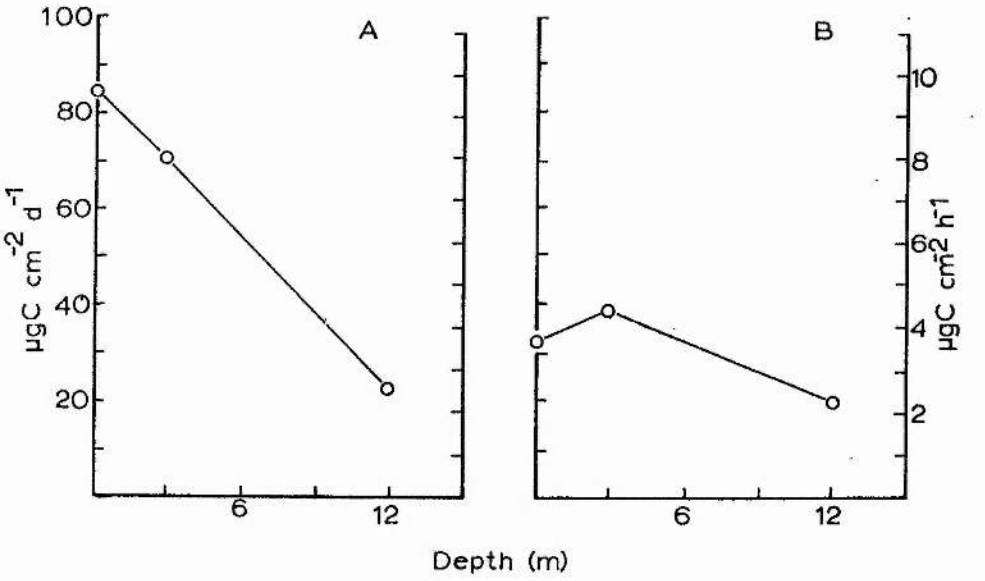


Figure 6.15. Rates of photosynthesis measured overnight at Dunstaffnage in situ (at 12m and after transfer to 3m and 0m; A, Dilsea; B, Phycodrys. Conducted in August; ^{14}C method; 13°C; 1430 to 1730h BST next day. Rates expressed per 24-hour day and also per daylight hour.

collected and incubated at 12 m in the clearer water at the Eilean Mor site where high efficiencies of 6.23% and 5.74% were achieved. Very high rates of fixation, on an extracted dry weight basis, were measured at 0 m in the finely branched littoral species Polysiphonia.

Figures 6.14 and 15 show the photosynthetic carbon fixation rates for certain "deep" growing (12 m) species incubated at their normal depth and also when transferred to 6 m, 3 m and 0 m. The curves for Dilsea (Figure 6.14B) are similar to those obtained at Puffin Island in that there was no inhibition of photosynthesis at 3 m. Fixation rates attained by Dilsea and Ulva at 6 m depth in the brown peaty waters at Rudha na Lain were almost as low as those measured at 12 m at Eilean Mor. Phycodrys, "green" Delesseria and Ulva all showed significantly higher rates at 3 m than at 12 m. The values obtained for "red" Delesseria were somewhat variable (Figure 6.14C) the mean rate at 3 m being lower, although not significantly so, than at 12 m.

Figure 6.15 shows the results of an experiment involving the incubation of Dilsea and Phycodrys overnight (total incubation time 27.17 h) in 450 ml bottles using the ^{14}C method. The rates (per daylight hour) were closely similar to those obtained in the short-term experiments. "Surface inhibition" was evident at 0m in Phycodrys but not in Dilsea.

c. Durness (^{14}C and oxygen methods)

Tables 6.7, 8 and 9 present the results of four experiments conducted on seven algal species at their natural depths of growth.

Table 6.7 Photosynthesis (^{14}C method) measured at Durness in June, temperature 9°C. Each determination is the mean of two replicates.

Species	Depth m	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Photosynthesis $\mu gC mg^{-1} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$	Efficiency %
Porphyra	0	22.70±3.08	6.47	92.8	1.02
Delesseria	9	2.88±0.15	0.48	13.5	0.89
Phycodrys	9	4.36±1.16	0.83	13.5	1.35

Table 6.8 Net photosynthesis (oxygen method) measured at Durness in June, temperature 9°C.

Species	Depth m	Photosynthesis $\mu l O_2 cm^{-2} h^{-1}$	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Photosynthesis $\mu gC mg^{-1} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$	Efficiency %
Phycodrys	9	1.61±0.06	0.87	0.92	9	0.40
Calophyllis	9	0.82±0.06	0.44	0.26	9	0.20

Table 6.9 Net photosynthesis (oxygen method) measured at Durness in October, temperature 11°C

Species	Depth m	Photosynthesis $\mu l O_2 mg^{-1} h^{-1}$	Photosynthesis $\mu gC mg^{-1} h^{-1}$	Photosynthesis $\mu gC cm^{-2} h^{-2}$	Irradiance J $cm^{-2} h^{-1}$	Efficiency %
Porphyra	0	1.52±0.19	0.82	2.34[a]	26	0.85
Laurencia	0	0.87±0.11	0.47	-	26	0.76
Rhodymenia	0	1.67±0.41	0.90	3.60[b]	26	0.33

[a] assuming SLA = 0.350 $cm^2 mg^{-1}$ [b] assuming SLA = 0.250 $cm^2 mg^{-1}$

From Table 6.7 it is seen that Porphyra attained the extremely high rate of carbon fixation of 22.7 $\mu gCcm^{-2}h^{-1}$ which is greater than any other in situ rate and the only occasion when a shallow incubation resulted in an efficiency value greater than 1%. The rate for Delesseria was less than found at Puffin Island and Dunstaffnage but that for Phycodrys was greater. The rate of carbon fixation attained by Phycodrys calculated from the oxygen method (Table 6.8) was about one fifth of the value attained by the ^{14}C method. Rates measured using the oxygen method in October, however, (Table 6.9), were of the same order as those attained by the same species at Puffin Island when the ^{14}C method was used.

When transferred to 0 m, Delesseria and Phycodrys (Figure 6.16) both showed significant inhibition of photosynthesis measured over a 3.5 h incubation period, compared with rates attained at the natural depth (Figure 6.16). When pretreated for 1 h in direct sunlight (no glass covering) no carbon fixation was recorded during the subsequent 3.5 h incubation; the implications of this will be more fully discussed in Chapter 7. In October, net photosynthetic rates (oxygen method) of the littoral species Porphyra, Laurencia and Rhodymenia were greatly reduced on transfer from 0 m to 15 m, Laurencia being close to compensation point (Figure 6.17).

d. Fife Ness (^{14}C method)

An experiment conducted in March (Table 6.10) yielded relatively low carbon fixation rates for Delesseria and Odonthalia collected at 9 m and incubated at 3 m. The value for Delesseria is similar to that attained by this species at 3 m at Puffin Island (Figure 6.10).

Table 6.10 Photosynthesis measured at Fife Ness in March, temperature 7°C, specimens collected at 9 m

Species	Depth m	$\mu gC cm^{-2} h^{-1}$	$\mu gC mg^{-1} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$ PAR	Efficiency %
Delesseria	3	1.72±0.55	1.38	36	0.20
Odonthalia	3	1.83±0.51	1.46	36	0.21

4. Discussion

Considering firstly the photosynthesis of algae incubated at their natural depths in the sea, Table 6.11 lists rates of photosynthesis determined in the sea by various workers from early experimenters (e.g. Gail 1922) to the present day. The table illustrates well the wide spread of values to be found in the literature and does not readily permit comparison of species, geographical bcation or other such variables. The rates of Goreau (1963) and Printz (1939) for instance are approximately one hundred times those of the present study and represent rates of over one hundred per cent per hour! Considering the wide range of values encountered in the present study, due to varying environmental factors, it seems probable that even for any one algal species, an average photosynthetic rate could only be

Table 6.11 Comparison of in situ rates of photosynthesis, expressed on area and dry weight bases

Species	Location	Depth m	Rate $\mu gCcm^{-2} h^{-1}$	Method	Author
Rhodymenia pertusa	Puget Sound	15	40.00	Oxygen	Gail (1922)
Polyneura sp.	" "	5	0.44	"	Tschudy (1934)
" "	" "	15	0.18	"	" "
Porphyra umbilicalis	S.E. Norway	0	6375.00	"	Printz (1939)
Macrocystis pyrifera	California	1	39.60	^{14}C	Clendenning & Sargent (1957)
Peyssonelia sp.	Jamaica	2	3700.00	"	Goreau (1963)
" rubra	Malta	50	5.00	"	Drew (1969)
Udotea petiolata	"	50	4.00	"	" "
Melobesioids	Marshall Is.	1	11.00	Oxygen	Marsh (1970)
Laminaria ochroleuca	Ganzirri	53	7.00	^{14}C	Drew (1972b)
" hyperborea	Fife Ness	5	18.00	"	Jupp (1972)
Posidonia oceanica	Malta	3	12.00	"	Drew & Jupp (1976
Posidonia oceanica	"	40	5.00	"	" " "
			$\mu gCmg^{-1} h^{-1}$		
Porphyra umbilicalis	S.E. Norway	0	2550.00	Oxygen	Printz (1939)
Delesseria sanguinea	"	6	1650.00	"	" "
Phycodrys sinuosa	"	2-4	1244.00	"	" "
Ulva lactuca	"	0	2775.00	"	" "
Peyssonelia sp	Jamaica	2	260.00	^{14}C	Goreau (1963)
Caulerpa prolifera	Canary Is.	0-15	0.38	"	Johnston (1969)
Dicyota dichotoma	" "	0-15	1.77	"	" "
Rhodymenia, Phycodrys & Delesseria	Cornwall	3	1.13[a]	"	Jupp (1972)
	"	3	0.03[b]	"	" "

[a] above, and [b] below L. hyperborea canopy

quoted in terms of an order of magnitude. Certain generalisations do emerge however, notably that in the present study no consistent difference was noted between photosynthesis measured in Mediterranean species and British species. Thus the photosynthesis of Ulva was similar at Ganzirri and Puffin Island (Tables 6.2 and 4). For Porphyra, however, the Ganzirri value (Table 6.2) was much higher than found at Puffin Island (Table 6.4) though similar to that recorded at Durness (Table 6.7). Thus the variation between sites in Britain was frequently as great as that between Mediterranean as opposed to British sites. The Ganzirri results may appear low because they mostly derive from the oxygen method (i.e. Table 6.1) but for example, Laurencia pinnatifida at Durness (Table 6.8) had a lower rate than L.obtusa at Ganzirri, both measured using the oxygen method. It should in fact be stressed here that photosynthetic rates attained at 53 m and 60 m at Ganzirri were of the same order as those at much shallower depths of 18 m at Puffin Island (c.f. Tables 6.2 and 4) and this, presumably, is in part attributable to the great water clarity at Ganzirri (although light levels were lower at 53 m at Ganzirri than 18 m at Puffin Island, assuming water at the former site to be Jerlov's Oceanic Type 2 - see Chapter 4). Considering the British sites alone, the results from the different locations were very comparable although comparison is somewhat confounded by the different month applicable to each site. Table 6.16 shows how rates for Delesseria at four different sites were remarkably similar, even although the months and irradiances were different; presumably in all cases, the algae were above saturation irradiance level.

Regarding photosynthesis with respect to taxonomic position, there was no consistent pattern of difference between the members of the Chlorophyta studied and members of the Rhodophyta from similar sites, using the same method and expressing photosynthesis in the same terms. Thus, similar rates were attained by shallow Laurencia and Ulva at Ganzirri (Table 4.1) and by

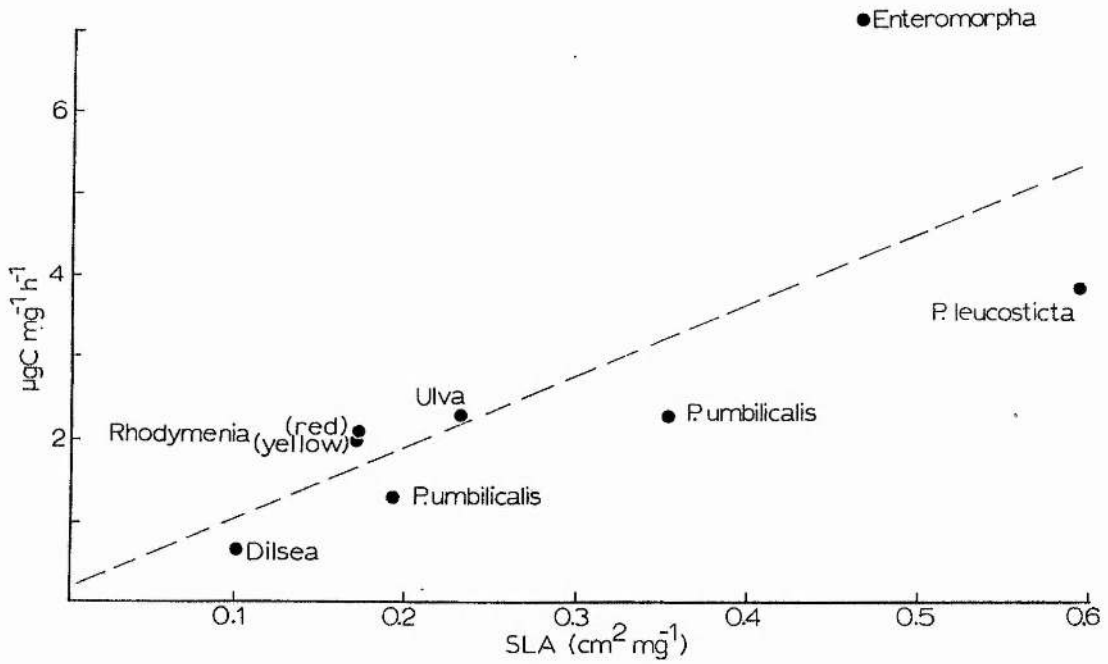


Figure 6.18. Fitted regression of photosynthetic rate (dry weight basis) upon SLA of several shallow British algae, from rates measured in situ (Table 6.4).

Rhodymenia, Ulva and Enteromorpha at Puffin Island (Table 6.4). This is similar to the findings of Bidwell (1958) who found no correlation between photosynthetic rate and taxonomic position in fourteen species of Rhodophyta, Chlorophyta and Phaeophyta studied in laboratory experiments, although Ulva lactuca was found to have the lowest rate of all, certainly not the case in the present study. Johnston (1969) found that photosynthesis in three species of Chlorophyta was significantly lower than in two species of Rhodophyta and six of the Phaeophyta growing in the same community at 15 m in the Canary Islands. Conversely, Kanwisher (1966) found that Ulva and Enteromorpha had higher rates than two species of Phaeophyta (Fucus vesiculosus and Ascophyllum nodosum) in laboratory experiments and concluded that photosynthesis was correlated more with thallus form than taxonomic position. Thus photosynthesis, considered on a dry weight basis, was necessarily lower in the more massive algal forms due to their low surface area per unit mass (i.e. low SLA). To find if a similar correlation existed in the species studied in the present work, the photosynthetic rates (on a dry weight basis) of the shallow species incubated in situ at Puffin Island (Table 6.4) have been plotted against SLA in Figure 6.18. Only the shallow specimens have been considered here, because the deeper algae may not have been fully light-saturated, which would further complicate the issue. There is a clear positive correlation between photosynthesis and SLA and the correlation coefficient of 0.75 was significant at the 5% level. The regression line drawn shows the underlying relationship. This finding is in agreement with the work of Kanwisher (1966) already mentioned. In a study of seven marine macroalgal species, Odum et al.(1958) found a close correlation between photosynthesis (on a dry weight basis) and surface-to-volume ratio, which is equivalent to SLA if equal density is assumed for all species.

Similarly, Littler (in press) found higher photosynthetic rates in sheet-like and finely branched species than coarser branched forms, and stated that no correlation was found between rates of photosynthesis and taxonomic position. However, having suggested that taxonomic position has little correlation per se, with photosynthetic rate, it should be pointed out that somewhat contrary to the theory of chromatic adaptation, the green alga Ulva in the present study maintained relatively high photosynthetic rates even when growing at and incubated at the deep sites. Thus, at Ganzirri (Table 6.1) Ulva had the highest rate at 53 m in the oxygen method experiments, on a dry weight basis, and a high rate also when the ^{14}C method was used (Table 6.2). At 18 m at Puffin Island, Ulva had the highest rate of all seven species studied growing naturally at this depth (Table 6.4). Photosynthetic rates attained by the phaeophyte, Laminaria hyperborea, in situ (Jupp 1972; Table 6.11) were not substantially different from those for the shallow algae of the present study, e.g. Rhodymenia, Porphyra and Ulva at Puffin Island (Table 6.4). At 18 m however, L.hyperborea had higher rates than Delesseria (Table 6.4) for example and again, this is contrary to the chromatic adaptation theory.

In comparative studies of British Laminarians, Jupp (1972) and Kain et al.(1975) suggested that the longer-lived perennial species (e.g. L.hyperborea) had lower in situ photosynthetic rates than "opportunist" annual species (Saccorhiza polyschides). In the present study, there was no evidence for such a trend when comparing the photosynthesis of species with conspicuous perennating organs, e.g., Rhodymenia (shallow) or Delesseria (deep) and the annuals or "pseudoannuals" with insubstantial perennating structures, e.g. Porphyra, Ulva, Enteromorpha (shallow), and Polyneura and Nitophyllum (deep).

The factor most clearly correlated with photosynthesis measured *in situ* in the present study was depth of growth. Markedly higher values were recorded for littoral and shallow sublittoral algae in Britain than for specimens growing and incubated in the deep sublittoral (Table 6.4). The more complete depth profiles at Ganzirri showed that marked optimum depths for photosynthesis occurred in the two species studied (Figure 6.1, Table 6.1). Although the higher respiration rate at 4.5 m and 15 m in *Ulva* might result in higher gross fixation rates at these depths, the rate at 4.5 m would still be comparatively low, and in any case there is little justification in assuming that dark respiration values represent the situation occurring in the light (see also Chapter 8). It seems likely that some form of photoinhibition is responsible for the low rates at shallow depths (especially in the case of *Sphaerococcus*, which only rarely cocurs at 4.5 m). The possibility that the lower rates at 4.5 m were due to an abnormal nutrient status in the shallow waters must not be overlooked. From the two-depth *in situ* experiments conducted in Britain there is no way of telling if optimum depths existed for algal photosynthesis. The fact remains that both shallow ecotypes in Britain and intermediate ecotypes at Ganzirri had relatively higher photosynthetic rates, and these findings are in agreement with the hypothesis that shallow and deep macroalgae are "sun" and "shade" adapted respectively, as suggested by Rabinowitch (1945), Biebl (1962) and, in phytoplankton, Yentsch (1962). In laboratory experiments, it has been shown that shallow (6 m) ecotypes of *Ptilota serrata*, *Phycodrys rubens* and *Chondrus crispus* (all Rhodophyta) had higher maximum photosynthetic rates than deep ecotypes (24 m) (Mathieson & Norall 1975).

It is of some significance that in all of the experiments utilising the oxygen method at natural depths, all algae were discovered to be above their compensation point for the duration of the incubation period (Ganzirri,

Table 6.1; Durness, Tables 6.8 and 9). Drew (1972b), in similar experiments, found that Laminaria ochroleuca was consistently at or below compensation point. Giaccone (1972) has suggested that this species may be heterotrophic, making use of nutrient-rich water brought in from the depths of the seas to North and South (Chapter 5), the absorption processes being aided by the strong currents which may help to reduce the thickness of the "boundering (sic) layer" a suggestion also put forward by Drew (1972b). Assuming that for a 12 h photoperiod, compensation is exceeded if net photosynthesis exceeds respiration (thus providing sufficient organic carbon for respiratory loss during the 12 h night), then considering the Ganzirri results (Tables 4.1 and 8.1) and the Durness results (Tables 6.8, 9 and 8.3), only the deep-water calcareous species Peyssonelia and Pseudolithophyllum seem likely to be below compensation point over a 24 h period. It thus seems less necessary to postulate heterotrophy in the species of the present study than it may be for L.ochroleuca. The problem of compensation in dealt with more fully in Chapters 8 and 9.

When considering the very different situation of the "transfer" experiments in which the degree of adaptation of individual "depth ecotypes" of species was being invesitgated, certain simple relationships with depth emerged. Firstly, the reduction in photosynthetic rate with depth noted in shallow species when incubated at deeper sites was related positively, although not always directly, to the reduction of irradiance with depth. Similar reductions in photosynthetic activity with depth have been noted by all other experimenters in the field (Gail 1922; Tschudy 1936; Printz 1939; Levring 1947, 1968, 1969) and the accepted view has been that the reduction is caused by the progressively lower sub-saturation (Chapter 7) irradiances encountered

with increasing depth. Although irradiance is probably the main factor acting in these short-term experiments, factors such as regular tidal exposure may be necessary features for certain eulittoral species (Doty & Archer 1950) and the decrease of water movement with depth may also be of importance (see Chapter 3). Hydrostatic pressure has been shown experimentally to be ineffective in altering photosynthetic activity (in Ulva lactuca and Delesseria sanguinea) until pressures equivalent to depths of the order of a thousand metres (i.e. well below the range of the photic zone) are reached (Shameel 1973). Levring (1947, 1968, 1969) showed that the reduction of photosynthesis, with depth, of transferred shallow species was frequently in direct relation to the reduction of irradiance i.e. the photosynthesis-depth curve was parallel to the light-depth curve. Such a situation was found to obtain in the case of Laurencia (Figure 6. 4) but the marked departure from this pattern shown by Ulva (Figure 6.6A) indicates that this species was saturated by irradiance between 4.5 m and about 30 m. Again, this finding is contrary to the chromatic adaptation theory which suggests that red algae are a priori better adapted for photosynthesis at great depths than green. It has already been noted that the littoral and upper sublittoral species tended to have higher rates of photosynthesis than the deep sublittoral algae, when both were incubated at their natural depths. The question remains whether, when the shallow species have been transferred to deep sites, their rates are lower relatively than those attained by the naturally occurring deep specimens. Table 6.12 shows the rates of photosynthesis of deep and shallow ecotypes of Sphaerococcus, Ulva and Dilsea when incubated at deep sites. In Sphaerococcus and Ulva, calculated on a dry weight basis, the rates

Table 6.12 Comparison of Photosynthetic rates of deep and shallow ecotypes of Sphaerococcus and Ulva (Ganzirri) and Dilsea (Puffin Island)

Species	Ecotype Source depth(m)	Incubation Site depth (m)	Photo-synthesis $\mu l O_2 mg^{-1} h^{-1}$	Ratio shallow/ deep %	Photo-synthesis $\mu g C cm^{-2} h^{-1}$	Ratio shallow/ deep
Sphaerococcus (oxygen method)	53	53	0.27	52		
"	4.5	53	0.14			
"	33	33	0.48	65		
"	4.5	33	0.31			
Ulva (oxygen method)	53	53	1.17	51	1.36	74
"	4.5	53	0.60		1.00	
"	33	33	2.38	56	2.75	81
"	4.5	33	1.33		2.22	
"	15	15	1.92	84	2.22	121
"	4.5	15	1.61		2.69	
" (^{14}C method)	53	53			3.85	45
" "	4.5	53			1.75	
Dilsea (^{14}C method)	18	18	2.60		2.60	107
" "	3	18	2.80		2.80	

attained at 15 m, 33 m and 53 m were higher in the shallow (source 4.5 m) specimens than the deep ecotypes. On an area basis however, due to the differential in SLA, Ulva from 4.5 m had a higher rate at the 15 m site than the 15 m ecotypes. These findings indicate that there do exist adaptive differences between shallow and deep ecotypes. In the case of relatively shallow transfers however, such as Ulva above (on an area basis) incubated at 15 m and Dilsea (source 3 m)

incubated at 18 m the relative performances of shallow and deep ecotypes may not be significantly different. Considering a wider range of species, some of which do not possess deep ecotypes, Table 6.13 shows the efficiences computed for shallow algae at Puffin Island, incubated at 18 m. All four values exceeded 2% and exceeded many of those for deep algae incubated in situ at 18 m (Table 6.4).

Table 6.13 Photosynthetic efficiency of shallow-water algae when transferred to deep (18 m) water (see also Figure 6.12)

Species	Depth m	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Irradiance J $cm^{-2} h^{-1}$ J PAR	Efficiency %
Porphyra leucosticta	18	2.93	4.28	2.89
Porphyra umbilicalis	18	2.35	4.40	2.24
Dilsea	18	2.63	2.88	3.82
Enteromorpha	18	3.25	4.24	3.21

Even the typically littoral green algae Enteromorpha, collected from a depth of 1 m below mean high tide level, had an efficiency exceeding that of several of the deep growing red species. These findings are contrary to the precepts of the theory of chromatic adaptation which suggests that these shallow-grown algae should be ill-adapted for photosynthesis in the very green light available at 18 m. One of the central assumptions of the chromatic adaptation theory (e.g. Rabinowitch 1951; Rabinowitch & Govindjee 1969) is that algae of the Rhodophyta should always be at a greater advantage than algae of the other algal divisions when incubated in deep water (especially coastal), due to the effective absorption of green light by the red pigment phycoerythrin.

A comparison of the relative reduction of photosynthesis in Puffin Island algae with and without phycoerthrin can be made by expressing photosynthesis at 18 m as a percentage of that at 3 m (Table 6.14). Algae which lack phycoerythrin are clearly the Chlorophyta, but also those rhodophyte specimens which have undergone bleaching by constant exposure to high irradiances in shallow water, namely Porphyra umbilicalis and Laurencia pinnatifida, at Puffin Island.

Table 6.14 Rates of Photosynthesis of shallow-water species incubated at 18 m at Puffin Island, expressed as a percentage of their shallow (3 m) rates (^{14}C method)

Species	Rate at 18 m / rate at 3 m %
Porphyra leucosticta (red)	46
Porphyra umbilicalis (yellow)	37
Laurencia pinnatifida (red)	35
Laurencia pinnatifida (yellow)	15
Dilsea	40
Enteromorpha	21

It can be seen that the least reduction was incurred in the two "reddest" Rhodophytes, Dilsea and P. leucosticta. The yellow form of Laurencia and the green alga Enteromorpha both underwent substantially greater relative reductions, whilst P. umbilicalis and the red form of Laurencia were intermediate. In the

absence of accurate pigment determinations, it is tentatively suggested that this gradation of response to incubation at 18 m is due principally to the gradation of the content of phycoerythrin, which is absent in Enteromorpha and typically reduced in "bleached" red algal thalli (see Chapter 5, p.150). Theoretically, the situation is slightly different in oceanic water types because they transmit more blue light which is effectively absorbed by the chlorophyll in the green algae. Thus, Levring (1968) found that the photosynthesis of Ulva lactuca was more greatly reduced by transfer to deep water in coastal areas than in oceanic water. Table 6.15 shows the photosynthetic rates of shallow-grown Ganzirri algae incubated at 53 m expressed as percentages of the corresponding rates at 4.5 m.

Table 6.15 Rates of photosynthesis of shallow-water (4.5 m) specimens of Ganzirri algae incubated at 53 m, expressed as a percentage of their rates at 4.5 m

Species	Rate at 53 m / rate at 4.5 m	
	Oxygen Method %	^{14}C method %
Laurencia	25	-
Gracilaria	67	16
Sphaerococcus	38	61
Ulva	40	49

In both the oxygen and ^{14}C methods, the degree of reduction in Ulva was less than in Enteromorpha at 18 m at Puffin Island (Table 4.14). In the oxygen method, the deep-red coloured Gracilaria had the highest relative rate at

53 m and the exposed and yellow-red Laurencia had the lowest rate. Caution should be exercised in such interpretations however, since the figure for Gracilaria using the ^{14}C method, although based on one experiment only, was the lowest value found. Broadly, however, these results indicate that (a) reduction of photosynthesis of both Rhodophyta and Chlorophyta is less at Ganzirri than in Britain (Puffin Island), and (b) the photosynthesis of chlorophytes may be reduced to a lesser extent at Ganzirri (due to blue oceanic-type water) than in Britain (Puffin Island, green coastal water).

From the experiments with Gracilaria (Figure 6.5A) and Dilsea and Ulva (Table 6.5) it appears that perhaps even more important than the water column in effectively attenuating irradiance and reducing photosynthesis in small-stature algae may be the shade produced by the canopy of the structurally dominant species in the community. These were Cystoseira sp. at Ganzirri and Laminaria hyperborea in Britain, of which the latter has already been shown to reduce irradiance substantially (Chapter 4). In areas where L. hyperborea is dominant in Britain, most of the non-laminarian growth occurs below the canopy either on the substrate or epiphytic on the stipes. The photosynthesis of Dilsea was reduced only to 22.6% of its value above the canopy whilst that of Ulva was drastically reduced to 1.5% comparing more closely with the figure of 2.8% found by Jupp (1972) for the red algae Rhodymenia, Delesseria and Phycodrys incubated at 3 m in Cornwall (Table 6.11). The greater reduction of Ulva at Puffin Island could be due to an alteration of the spectrum by absorption by the L. hyperborea fronds as suggested by Kitching (1941). It would be expected that brown algae would be similarly affected, however, and this was not borne out by Jupp (1972) who found that, in his experiment mentioned above, the photosynthesis of L. hyperborea was reduced to 4.7% below the canopy, and in other experiments photosynthesis of L. hyperborea was reduced to 14 - 63% and Saccorhiza polyschides 13 - 51%

of their respective values above the canopy. In biomass studies, Kain (1976) found that although the Chlorophyta (mostly Ulva) composed a maximum of 0.02% of virgin L. hyperborea forest at the Isle of Man, the proportion could rise to a maximum of 62% in areas artificially cleared of L. hyperborea, suggesting a possible selective effect of the canopy on the Chlorophyta. Returning to the photosynthesis experiments, it is possible that increased irradiance due to the movement of the canopy fronds during turbulent water conditions (experiments were carried out during calm weather) may result in days of high and low photosynthesis below the canopy, resulting in a net increment over an extended period.

Considering the transfer of deep-living specimens to shallower sites, a variety of responses was noted. Specifically, algae exposed to the highest surface (0 m) irradiances available, such as Peyssonelia and Pseudolithophyllum (Figure 6.9) at Ganzirri, Delesseria and Phycodrys at Durness (Figure 6.16) and certain species incubated at 3 m at Puffin Island (Figure 6.13) showed markedly reduced rates compared with deeper incubations at normal sites of growth. Similar effects have been reported by Gail (1922), Printz (1939) and Levring (1947) in sublittoral species from all three major algal divisions. Such a reduction of photosynthesis in phytoplankton studies has been recognised since the pioneer work of Marshall & Orr (1928) and is referred to as surface inhibition or photoinhibition. Gail (1922), Printz (1939) and Levring (1947) also noted that surface inhibition was less likely to occur in cloudy weather, a feature confirmed by comparing the results from Puffin Island (weather predominantly sunny) with those from Dunstaffnage (cloudy weahter) and which strongly implicates high irradiance as the prime factor. The response of algae to the changing irradiance produced by the

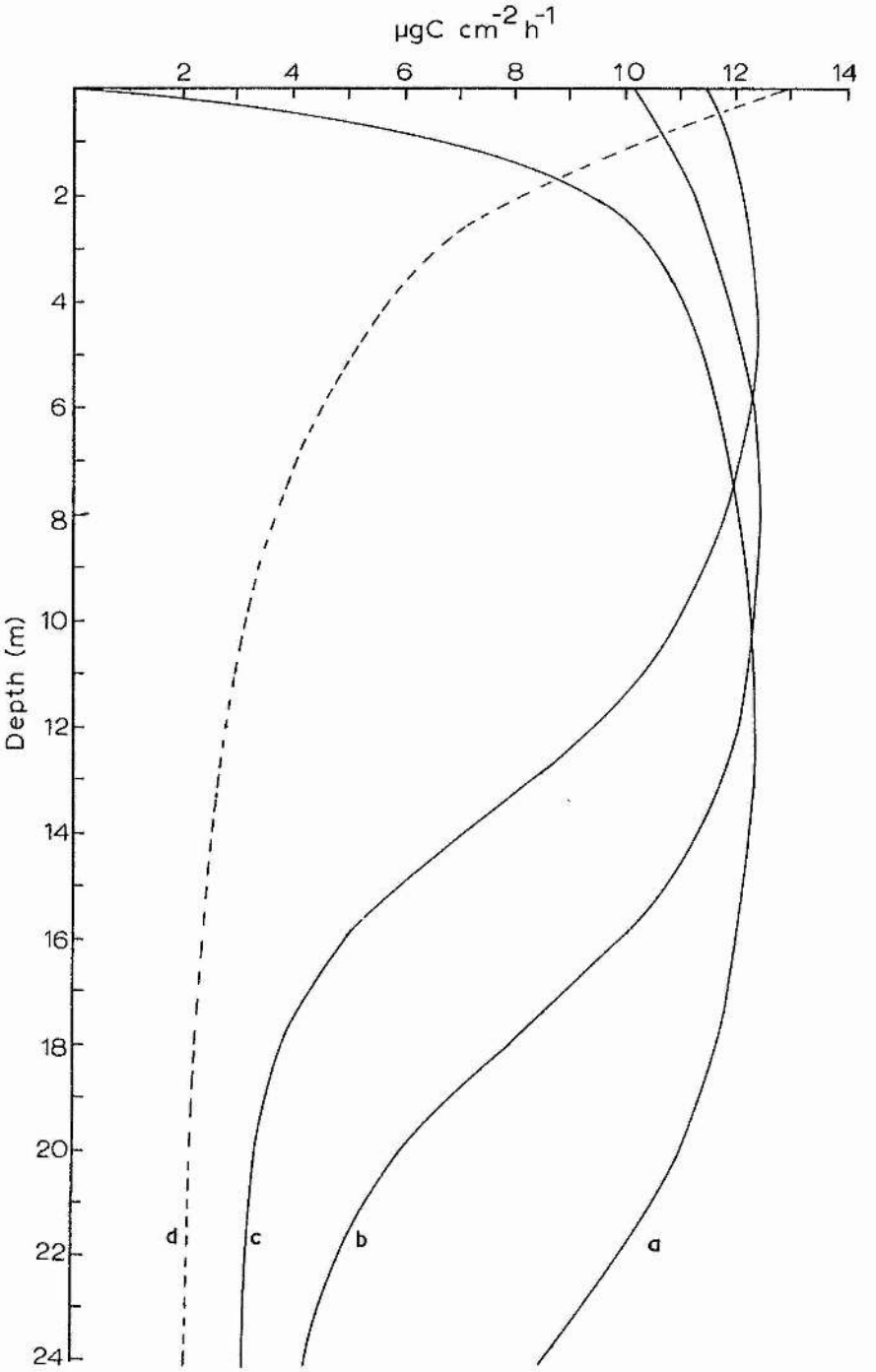


Figure 6.19. Relationship of photosynthesis to depth, in Delesseria, for surface irradiance levels of a, 30 , b, 15 and c, 7.5mW cm^{-2} PAR (computed from Figure 7.20). Attenuation of irradiance is shown by curve d .

interaction of depth and surface irradiance can be illustrated by plotting an empirical photosynthesis-irradiance curve (Chapter 7) as a photosynthesis-depth curve. This has been done for Delesseria in Figure 6.19 (computed from Figure 7.20). Curve (a) shows the vertical profile of Delesseria (collected at 18 m, Puffin Island) for a sunny day (30 $mWcm^{-2}$ PAR). Photo-inhibition is greatly in evidence at the surface and down to 6 - 8 m, then maximum photosynthesis continues to around 20 m, with a wide optimum depth range of about 12 m. As surface irradiance decreases through 15 to 7.5 $mWcm^{-2}$, the optimum depth decreases to 8 and then 5 m, and surface photoinhibition is almost absent. Below 20 m depth in curves (b) and (c) and at even greater depth in curve (a), the photosynthesis curve begins to parallel the irradiance attenuation curve (d) indicating that irradiance is limiting photosynthesis directly, and as surface irradiance decreases the depth at which limitation occurs becomes shallower. Laurencia at Ganzirri (Figure 6.4) was an example of an alga whose photosynthesis was directly limited by irradiance for the full length of the 4.5 m to 53 m profile, and the corresponding curve for Ulva (Figure 6.6A) resembles curve (c) in Figure 6.19. (A more detailed discussion of the relationship between surface irradiance and photosynthesis in the depth profile is given in Chapter 10.)

The fact that Peyssonelia and Pseudolithophyllum underwent less photoinhibition when incubated at 0 m for 1.5 h as opposed to 5 h (Figure 6.9) points to the dose-rate dependence of this phenomenon. Thus, even Dilsea which showed an apparent resistance to photoinhibition in each experiment, including the 27 h incubation period (Figure 6.15A) may still have a threshold does which requires a longer exposure to high irradiance than do Phycodrys or Delesseria, for example. (Again, this aspect is dealt with

further in Chapter 7.) Although no experiments were conducted to assess the affect of the L. hyperborea canopy on photosynthesis or photoinhibition of deep algae incubated at shallow sites, it seems likely that the canopy would reduce photoinhibition. In this manner, the shade of the canopy may extend the upper colonisation limits of shade-loving algal species.

Neither the single experiment conducted at Ganzirri to investigate a possible photosynthetic daily periodicity, nor the routine experiments conducted at Ganzirri and in Britain revealed any gross differences between photosynthesis in morning and afternoon which were not directly related to irradiance. Daily periodicity of photosynthesis has been reported in phytoplankton by Doty & Oguri (1957) indicating morning (10.00 h GMT) maxima and afternoon (19.00 h GMT) minima which are not otherwise correlated with inhibitory or sub-compensation irradiances. Pomeroy (1959) however suggested that phytoplankton photosynthesis declined after noon as a result of prolonged exposure to bright light. In this connection, Yentsch (1963) correlated reduction of phytoplankton photosynthesis in late afternoon with a concurrent reduction in chlorophyll content ascribed to the photo-oxidation effects occurring at high irradiances. The well-known diurnal periodicity of photosynthesis occurring in terrestrial plants is connected with stomatal opening and closure and thus involves a different mechanism (Heath 1969).

The marked seasonal changes in photosynthetic rates (both oxygen and ^{14}C methods) noted at Ganzirri in Ulva and Sphaerococcus may be correlated with a spring burst of growth. When expressed on an extracted dry weight basis, the results for Ulva showed a high seasonal variation which was apparently due to a seasonal variation in the proportion of the alcohol soluble component of the thallus. Fluctuations in photosynthetic capacity between the months of August and December have been noted in the

red Wrangelia the Adriatic Sea (Zavodnik 1973) but there was no clear correlation with season. The British species for which most seasonal data were available was Delesseria, and Table 6.16 shows values of photosynthesis measured for this species during different months at various sites. The values are remarkably consistent but in any case, any seasonal variation would be inseparable from possible geographical variations.

Table 6.16 Photosynthesis in Delesseria, on a seasonal basis (^{14}C method) at different British sites

Month	Photosynthesis $\mu gC cm^{-2} h^{-1}$	Irradiance $J\ cm^{-2} h^{-1}$ PAR	Depth m	Site
March	1.72 ± 0.55	36.00	3	Fife Ness
June	2.88 ± 0.15	13.50	9	Durness
July	3.05 ± 0.57	3.78	18	Puffin Island
August	3.77 ± 0.90	7.19	12	Dunstaffnage

Although it is probable that in all cases the plants were incubated above saturation irradiance (i.e. 1-2 $mWcm^{-2}$ PAR, see Chapter 7), it may be that the low value at Fife Ness in March was due to photoinhibition, since the material was collected at 9 m and incubated at a depth which, due to the tide, varied from 1.5 m to 4.5 m; variation in the photosynthesis of temperate algae have been investigated at Helgoland (Luning 1971) and photosynthetic activity found to have a maximum in March at low irradiance (4 $mWcm^{-2}$) and

in August at high irradiance (17 $mWcm^{-2}$). In *in situ* experiments, however, Jupp (1972) found that photosynthesis in *L. hyperborea* was at a maximum in spring and substantially lower in summer. Such a pattern has been explained in terms of the summer depletion of nutrients such as phosphorus and nitrogen (Black & Dewar 1949) which similarly depress phytoplankton production in summer (e.g. Raymont 1963).

CHAPTER 7

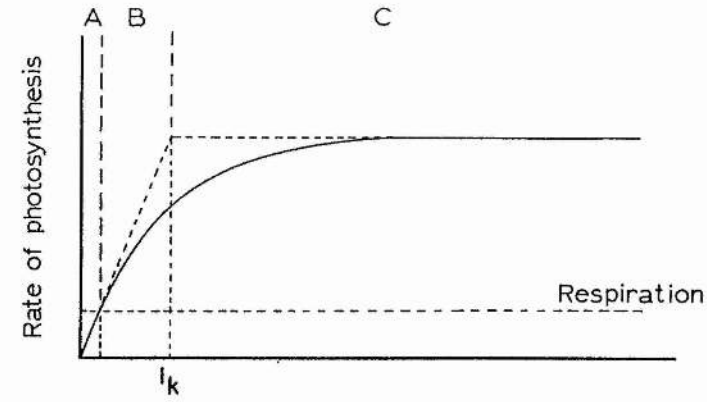
The relationship between photosynthesis and level of irradiance

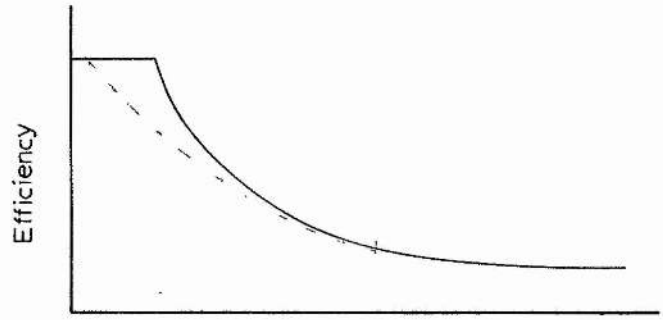
CONTENTS

1. Introduction

Together with supply of inorganic carbon, supply of radiant energy is one of the major external factors normally limiting photosynthetic rate in plants. The marine environment contains a wide range of irradiances both in terms of quality and quantity, as described in Chapter 4. The interaction of plants with different qualities and quantities of irradiance was extensively reviewed by Rabinowitch (1945, 1951, 1956). The adaptation of higher plants to different light environments has been studied by Gabrielsen (1948), Björkman & Holmgren (1963) and by contributors to Light as an ecological factor, I (Bainbridge et al. 1966) and II (Evans et al. 1975). Responses of marine phytoplankton to differing irradiance regimes have been studied by Ryther (1956) Steemann-Nielsen (references in Steemann-Nielsen 1974) and Jitts et al. (1964) and of marine macroalgae by Montfort (1929) Stocker & Holdheide (1938), Levring (1947), Gessner (1955) and, more recently, Kanwisher (1966), Mathieson & Burns (1871), Mathieson & Norall (1975) and Kain et al. (1976).

The present chapter deals principally with the relationship between photosynthesis and quantity of radiation, although the implications of the possible effects of different spectral qualities will be considered in





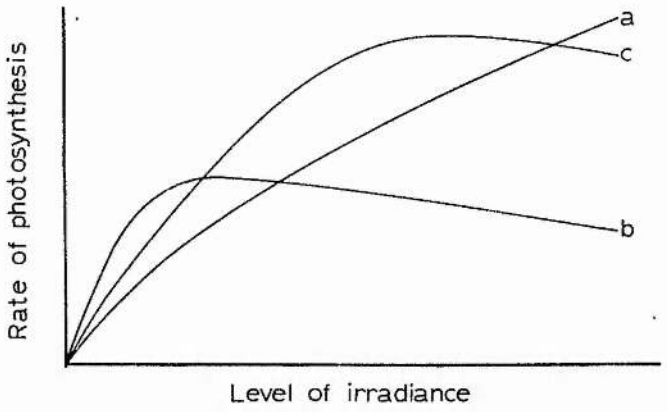


Figure 7.1. (upper). Basic form of photosynthesis - irradiance curve; A, region below compensation irradiance; B, region of "light limitation"; C, region of "light saturation". I_k, saturation irradiance.

Figure 7.2. (middle). Exponential drop in efficiency at irradiances greater than I_k.

Figure 7.3. (lower). Photosynthesis - irradiance curves; a, "sun"plant; b, "shade" plant; c, intermediate plant (redrawn from Bidwell, 1974).

the discussion. Figure 7.1 shows the basic form of the photosynthesis-irradiance curve and its three main regions. At very low irradiances the fixation of carbon is less than the rate of loss of carbon due to respiration and the plant is said to be "below compensation point" (region A in Figure 7.1). At the "light compensation point" the irradiance is sufficient to support carbon fixation exactly equal to respiratory loss. Respiration is indicated in the figure as a dotted line, but this is an over-simplification, as it is by no means certain that respiration in the light remains constant in increasing irradiance levels (e.g. Heath 1969). Just above the compensation point is a zone where photosynthesis is more or less proportional to irradiance, giving a linear relationship known as the region of "light limitation" (B in Figure 7.1). This region merges slowly into a plateau where further increase in irradiance has no further effect on photosynthesis, the region of "light saturation" (C in Figure 7.1). In this region, photosynthesis is saturated by light and limited by some other factor or factors such as availablility of inorganic carbon or enzymes within the photosynthetic mechanism. Although there is, strictly, no clear transition between B and C, these portions of the curve can be extrapolated as shown, their point of intersection being the "saturation point", designated I_k by Talling (1957). Not shown on the curve is the condition known as "photoinhibition", a reduction in photosynthesis occurring at high irradiances, at the distal end of zone C in Figure 7.1.

Because zone A-B is generally a curve, it is steepest at low irradiances, near the compensation point, and thus in this region, the plant usually reaches its highest photosynthetic efficiency, the ratio of units of energy fixed to units of energy available. Efficiency decreases exponentially with increase in irradiance above I_k (Figure 7.2).

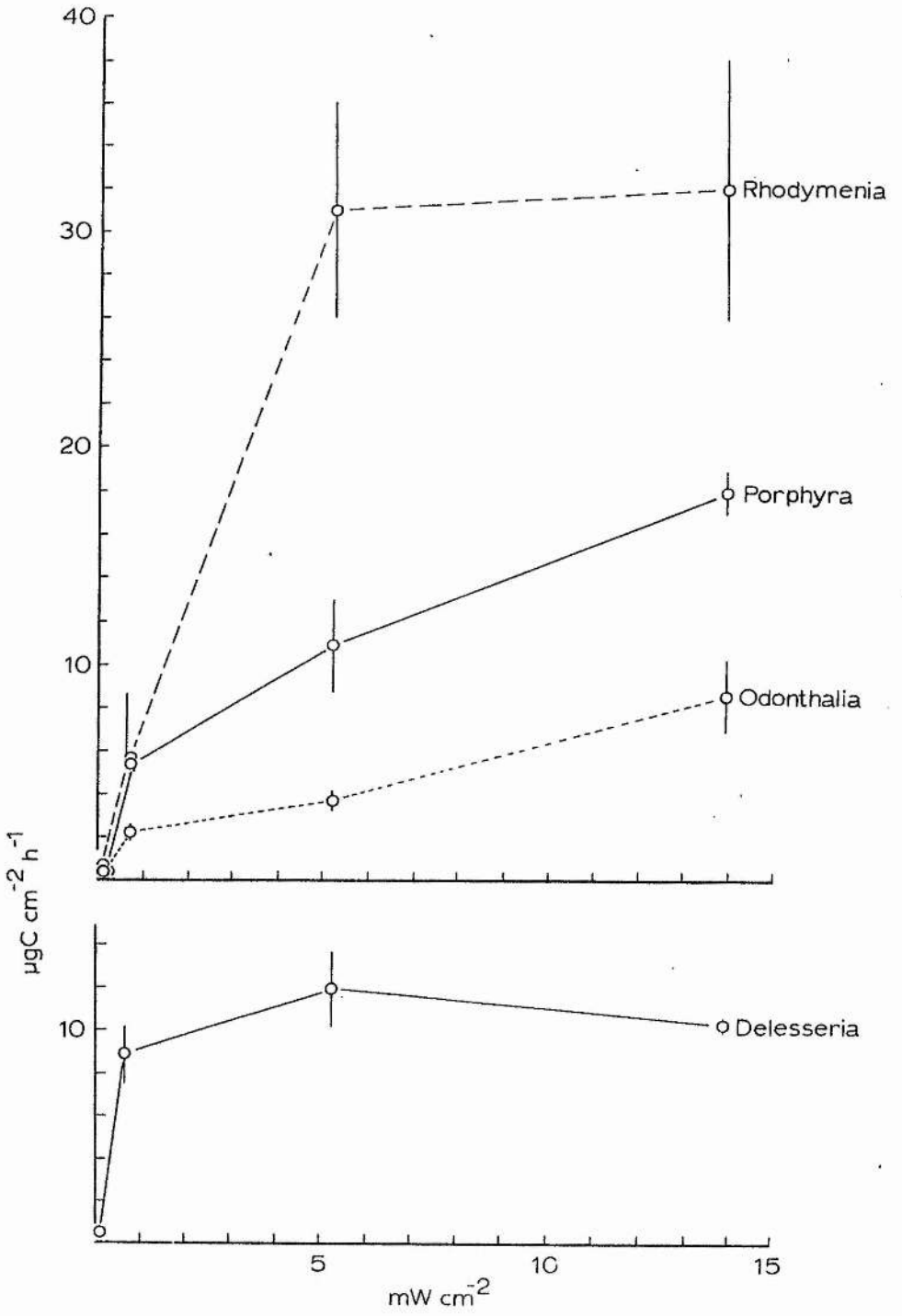


Figure 7.4. (upper). Photosynthesis - irradiance curves measured in the laboratory for three algal species collected at Fife Ness; conducted in March; ^{14}C method; 11°C; 1h.

Figure 7.4. (lower). As above, for Delesseria

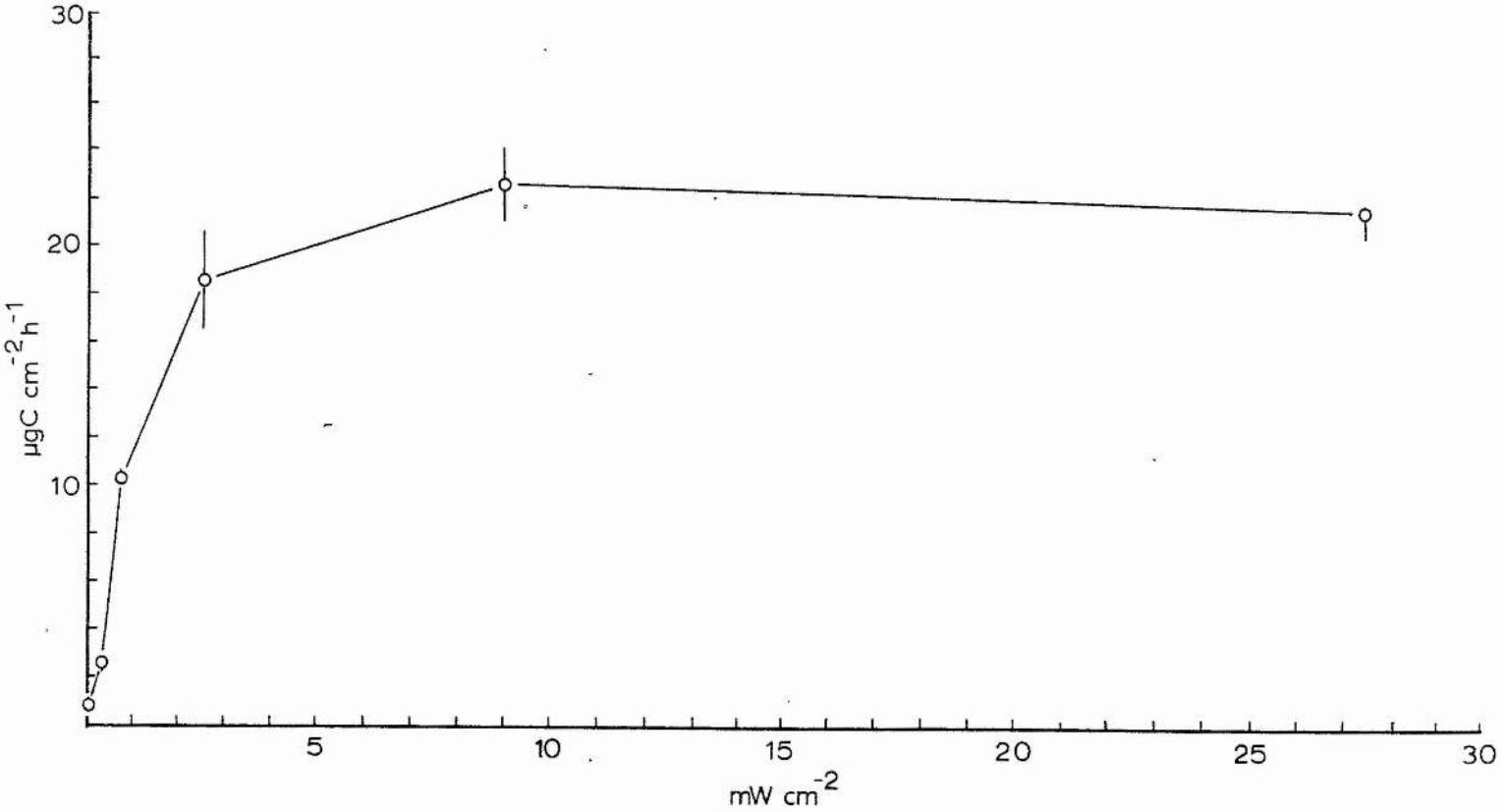


Figure 7.5. Photosynthesis - irradiance curve measured in the laboratory for Dilsea collected at Fife Ness. (Conditions as for Figure 7.4.).

It is well established that plants living under different conditions of irradiance typically show different responses to increases in irradiance. Figure 7.3 shows three curves representative of the response of plants adapted to sun (a), shade (b) and intermediate (c) environments (Bidwell 1975). The situation is extremely complex, since phenotypical adaptations by ecotypes of individual plant species and strains, together with genetic adaptations between species, produce an almost infinite number of variants of these curves between the extremes shown by (a) and (b). It was suggested by Montfort (1929), Stocker & Holdheide (1938) and, later, Mathieson & Burns (1971) that littoral algae might be sun-adapted, and sublittoral algae shade-adapted, and the results in the present chapter have been discussed in this context.

2. Tungsten-iodide light source

Five experiments were conducted in the laboratory to investigate the relationship between irradiance and photosynthesis measured by the ^{14}C method. Four of these, on Rhodymenia, Porphyra, Odonthalia and Delesseria were carried out using the apparatus described in Chapter 2 (p. 23) providing four levels of irradiance (maximum 14 $mWcm^{-2}$ PAR). A fifth experiment, conducted on Dilsea, utilised the improved incubation apparatus (Chapter 2, p. 24) allowing six irradiance levels to be used, with a maximum of 28 $mWcm^{-2}$ PAR. The results are shown graphically in Figures 7.4-6. The shapes shown by the photosynthesis-irradiance curves encompass all three of the examples shown in Figure 7.3. Thus Porphyra (littoral) and Odonthalia (sublittoral, 9m) had curves of the "sun" type, Rhodymenia (shallow sublittoral) had an "intermediate" type (Figure 7.4)

and Dilsea and Delesseria (both sublittoral, 9 m) had curves of the "shade" type (Figures 7.5 and 6). It is not known, however, if Dilsea would have shown inhibition at 14 mW if measured at this irradiance. All of the curves show similar extremely steep slopes at low irradiance (i.e. less than 1 $mWcm^{-2}$) unlike the examples in Figure 7.3. Thus the curves for Porphyra and Odonthalia each consist of two distinct zones, each a straight line, implying proportionality of photosynthesis to irradiance, and also implying a high saturation irradiance (i.e. greater than 14 $mWcm^{-2}$). The curve for Rhodymenia is steeply linear to 5 $mWcm^{-2}$ at which irradiance the material was apparently almost saturated. The curves for Delesseria and Dilsea both show steep rises to relatively high rates within 1 mWcm followed by a small slope leading to maxima at 5 mW (Delesseria) and 7 mW (Dilsea) thereafter decreasing.

It is notable that the maximum carbon fixation rates attained in these experiments were extremely high compared with the in situ rates (Chapter 6).

3. Surface Sunlight

(a) Experiments at British sites

Experiments were conducted at Puffin Island and at Dunstaffnage using freshly collected algal tissue from the in situ sites. Tissue was incubated at four levels of irradiance using the apparatus described in Chapter 2, p.23, and the oxygen method was used to determine rates of photosynthesis and dark respiration. Experiments were carried out on

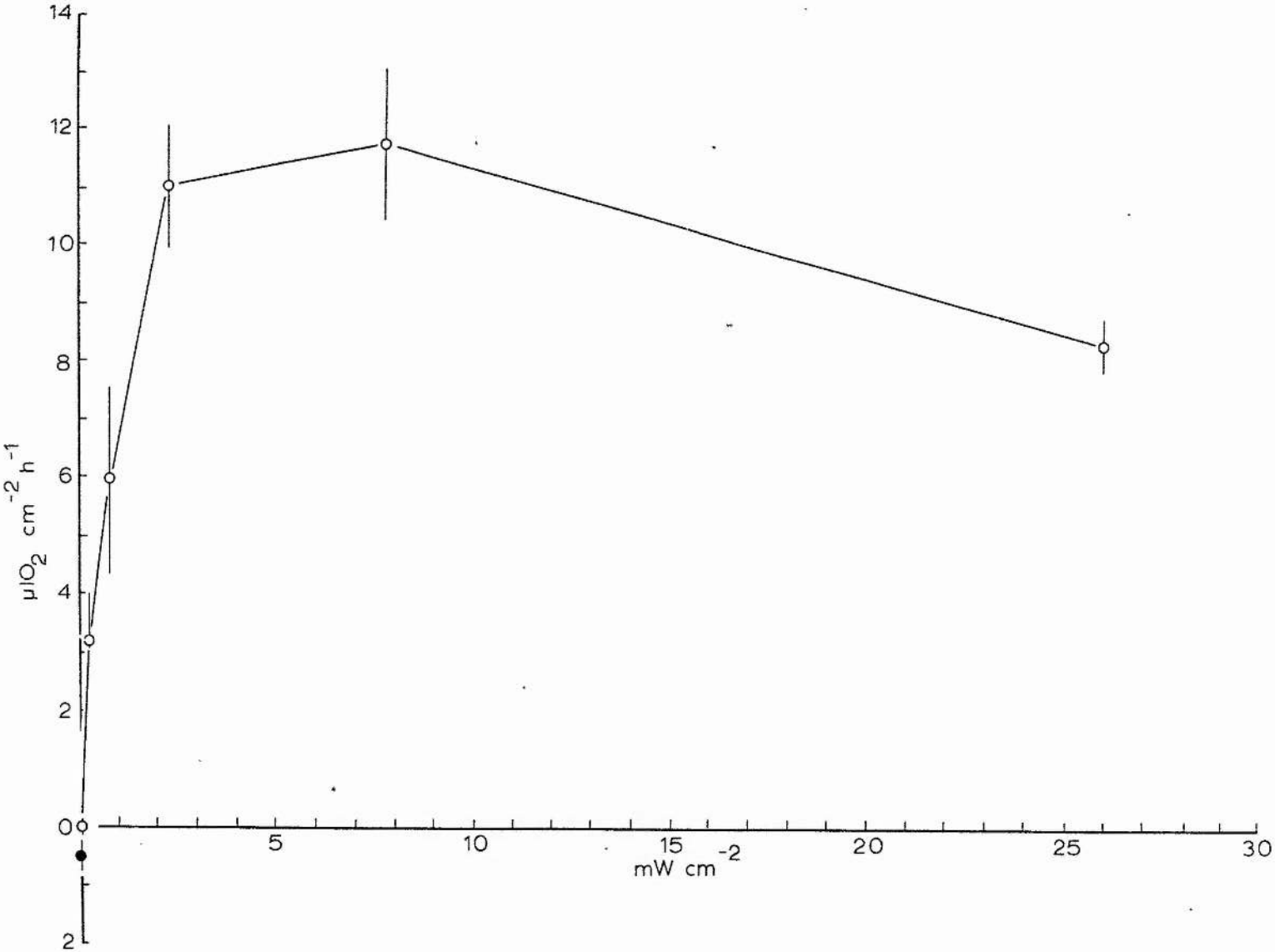


Figure 7.7. Photosynthesis - irradiance curve measured in surface sunlight for Rhodymenia at Puffin Island; conducted in July; oxygen method; 18.5°C, 2h. Filled symbol represents dark respiration rate.

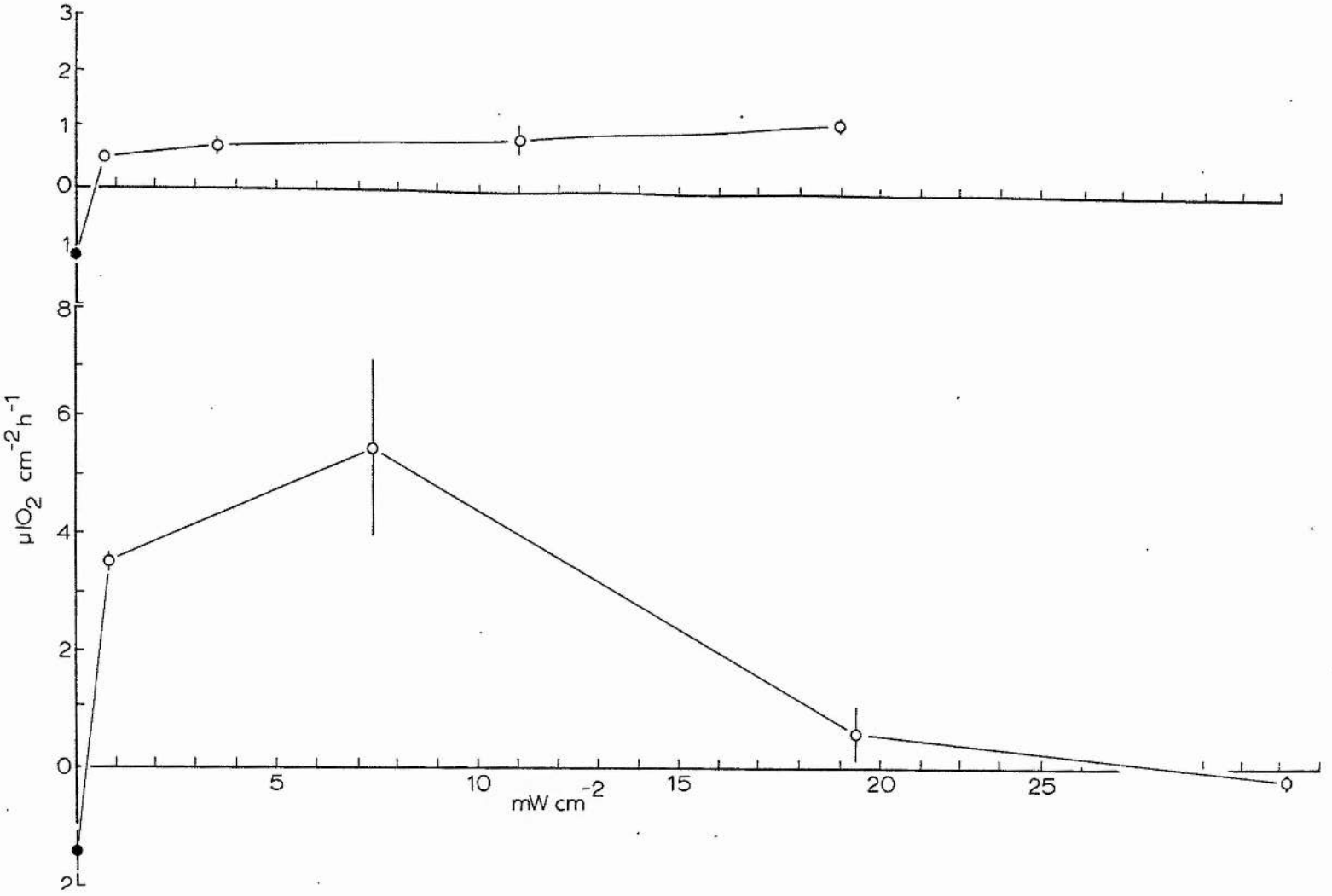


Figure 7.8. (upper). Photosynthesis - irradiance curve of Polyneura (source 4.5m) measured in surface sunlight at Puffin Island; conducted in July; oxygen method; 18°C; 2h. Filled symbol represents dark respiration rate.

Figure 7.9. (lower). As above for Polyneura, source 18m.

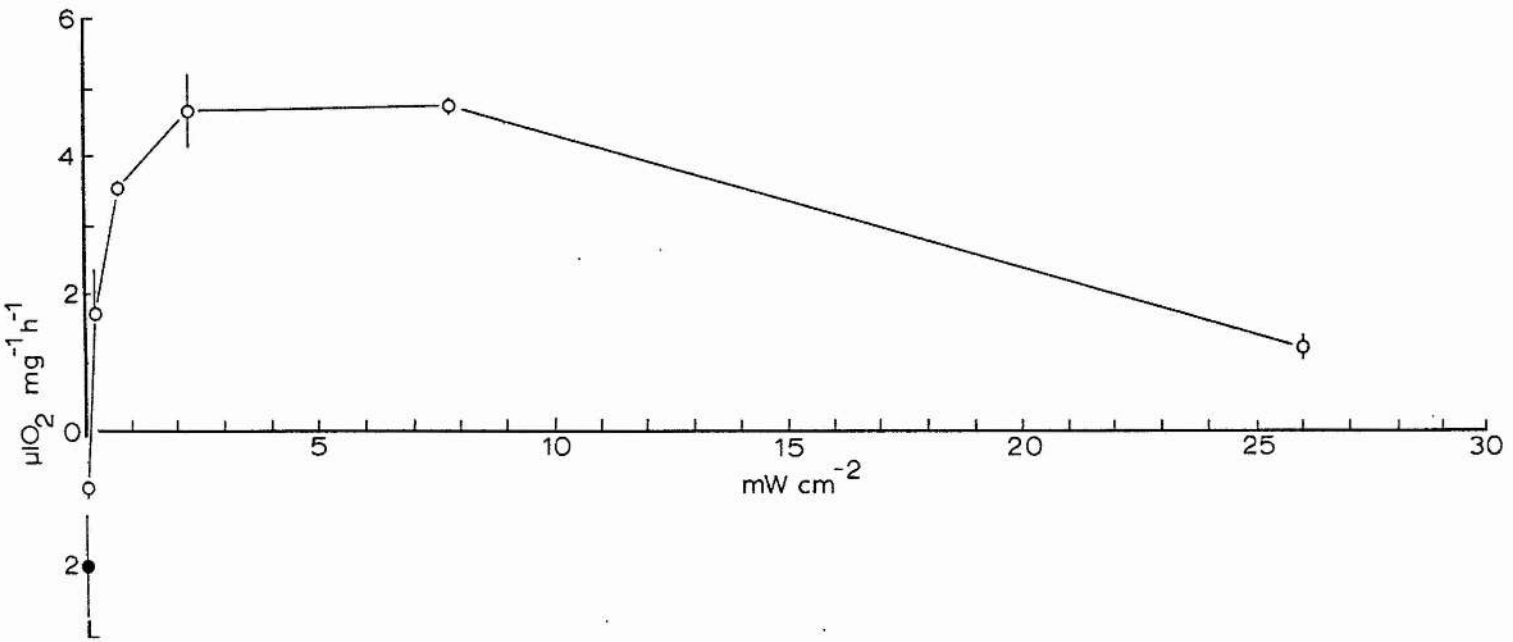


Figure 7.10. Photosynthesis - irradiance curve of Polyneura (source 18m) measured in surface sunlight at Puffin Island (following year from Figure 7.9.); conducted in July; oxygen method; 18.5°C; 2h. Filled symbol represents dark respiration rate.

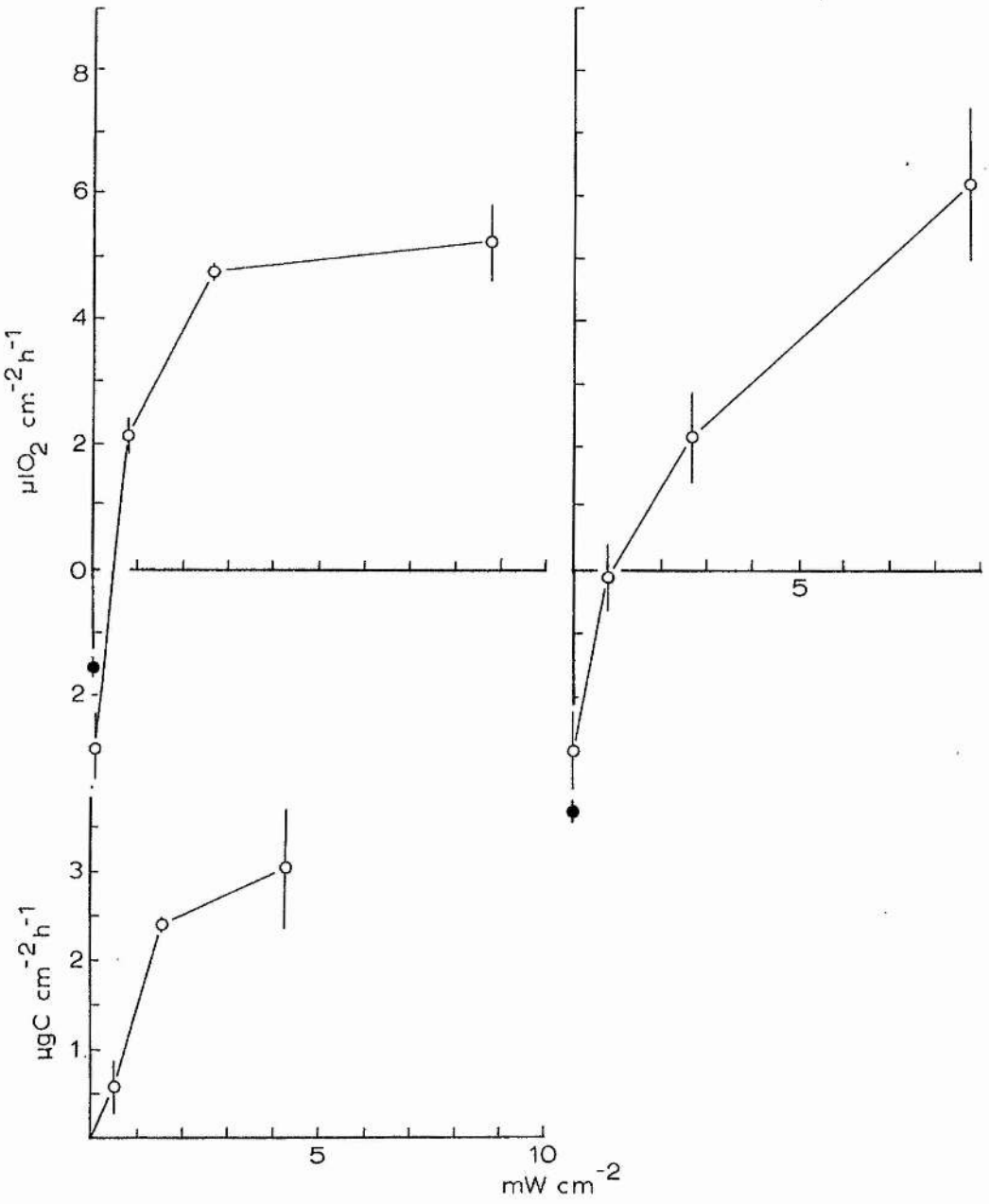


Figure 7.11. (upper left). Photosynthesis - irradiance curve of Delesseria (source 6m, red tissue) measured in surface sunlight at Dunstaffnage; conducted in August; oxygen method; 16°C; 2h. Filled symbol represents dark respiration rate.

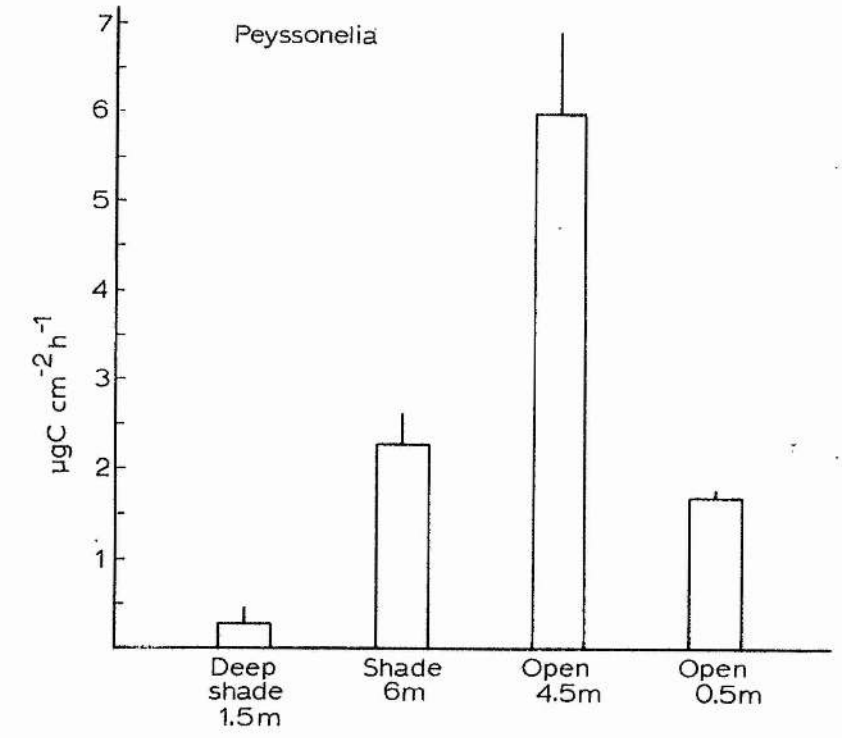
Figure 7.12. (upper right). As above, for Delesseria, source 6m, green tissue.

Figure 7.13. (lower). Photosynthesis - irradiance curve for Delesseria (source 9m, Fife Ness) measured in surface sunlight at St. Andrews; conducted in May; ^{14}C method; 11°C; 2h.

Rhodymenia (upper sublittoral, epiphytic on L.hyperborea) as a representative of the shallow water flora, (Figure 7.7); Polyneura from 4.5 m and 18 m, to investigate the characteristics of these depth-distinct ecotypes, (Figures 7.8, 9 and 10); Delesseria from 6 m, both normal red thallus and green "bleached" thallus, to investigate a possible difference in photosynthetic capacity in these two forms from one depth (Figures 7.11 and 12). Also included (Figure 7.13) are the results of one experiment carried out in sunlight, at St Andrews, on Delesseria (source 9 m) using the incubation chamber from the laboratory system (Chapter 2, p.23).

As in the laboratory experiments, Rhodymenia had the highest rate at saturation, but here, photoinhibition was apparent at the highest irradiance, 26 $mWcm^{-2}$ (Figure 7.7). The curves obtained for Polyneura (source 18 m) at irradiances below 10 mW were closely similar, although obtained in different years (Figures 7.9 and 10). Above 10 $mWcm^{-2}$, both showed photoinhibition which resulted in no net oxygen evolution in one experiment (Figure 7.7). The curve for Polyneura from 4.5 m, collected below the L. hyperborea canopy indicated a saturation irradiance of 1-2 $mWcm^{-2}$ (Figure 7.8), but the maximum photosynthetic rate attained (at 19 $mWcm^{-2}$) was only about one-fifth of that for the material from 18 m (attained at 10 $mWcm^{-2}$, see Figure 7.9). The curve for Delesseria with the red thallus showed that saturation occurred at from 1-3 $mWcm^{-2}$ but photoinhibition was not apparent at the lower maximum irradiances obtaining at Dunstaffnage (Figure 7.11). The green thallus material had a markedly contrasting relationship to irradiance, showing no indication of saturation up to 9 $mWcm^{-2}$ (Figure 7.12). The St Andrews experiment on this species showed results similar to those for red thallus at Dunstaffnage (Figure 7.13).

An interesting phenomenon was exhibited by the red thallus material of Delesseria at Dunstaffnage where the tissue incubated at an irradiance of 0.07 m Wcm^{-2}, which was below compensation, apparently took up more oxygen than the tissue used for the measurement of dark respiration under otherwise identical conditions (Figure 7.11).



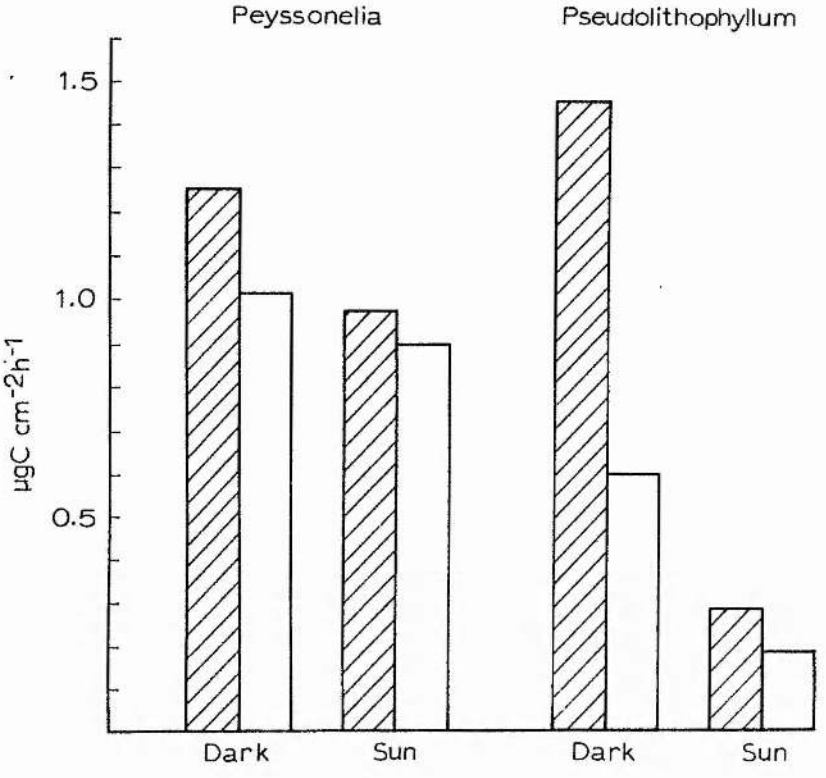


Figure 7.14. (upper). Rates of photosynthesis of Peyssonelia at Marsala, showing lowered rates in deep shade (1.5m) and shade (6m) due to sub-optimal irradiance levels and also in the open (0.5m) due to photoinhibition by supra-optimal irradiance levels; conducted in August; ^{14}C method; 25°C; 4.5h (1030 - 1500 h BST).

Figure 7.15. (lower). Rates of photosynthesis of Peyssonelia and Pseudolithophyllum after "dark" and "sun" pretreatments; incubated in the shade, 0.4 mW cm^{-2} PAR (hatched columns) and in full sunlight, 36 mW cm^{-2} (open columns). Experiment conducted in April; ^{14}C method; 14.5°C; pretreatment 1h, incubation 5h (1000 - 1500 h BST).

4. Photoinhibition

A reduction of photosynthetic rate induced by high irradiance has already been noted in deep-growing algae when transferred to shallow depths (Chapter 6) and also in the course of light saturation investigations (see earlier sections of this chapter). The following experiments further investigated the nature of photoinhibition with reference to both quantities and quality of irradiance involved.

a. Mediterranean algae - Marsala site.

Two experiments of a preliminary nature were carried out on Peyssonelia sp. which was collected from areas of dense shade beneath rocks at a depth of 2.2 m. The irradiance was not measured, but an approximation has been made from the data of de Jong (1973); the conditions were full sun, clear skies throughout, water temperature 25°C. The ^{14}C method was used, performed in situ, and four incubation treatments were involved, (1) relative shade, beneath a Posidonia sea-grass bank at 6 m depth, (2) open water at 4.5 m, (3) unshaded, on rock surface, depth 0.5 m, and (4) deep shade beneath a rock, 1.5 m depth. The results are presented in Figure 7.14, with ambient irradiance values. Optimal conditions appeared to be at 4.5 m depth in open water, with an irradiance of $\sim$ 17 $mWcm^{-2}$. Extreme photoinhibition was shown by the material incubated at $\sim$89 $mWcm^{-2}$.

Ganzirri site

An experiment was conducted in April on the deep-water algae Peyssonelia and Pseudolithophyllum collected at 60 m depth. Samples were stored overnight in the dark at 14.6°C and immediately prior to the experiment, sub-samples were exposed to 1h full sunshine (at 0900 BST, giving 16.6 $mWcm^{-2}$ PAR) covered by only 5 cm seawater at 15°C (the samples were not under glass).

Other sub-samples were incubated concurrently in the same tank, enclosed in black polythene to exclude light. Samples were subsequently placed in incubation bottles and exposed for 5h to either full sunlight (mean irradiance approximately 36 $mWcm^{-2}$ PAR) or dense shade (mean irradiance approximately 0.4 $mWcm^{-2}$ PAR) produced by incubating within the aquarium building. The light data were extrapolated from de Jong (1973) and subjective observation. The temperature was maintained at $15\pm1^{\circ}C$ by the addition of ice to the seawater bathing the incubation bottles. Photosynthesis was measured using the ^{14}C method, and the results are presented in Figure 7.15. In both species the highest rates were attained by the dark-pretreated material incubated at low irradiance, and minimum rates attained after light pretreatment and incubated at high irradiance. The reduction of photosynthetic rate was related positively, although not directly, to the increase in energy dose. Pseudolithophyllum showed a much greater response to high irradiance than did Peyssonelia. This experiment can be compared with that deccribed in Chapter 6 (Figure 6.9) which shows essentially similar features.

b. British algae - Durness site

Two experiments were conducted in June in natural sunlight conditions. The first involved the sublittoral species Delesseria and Nitophyllum (both from 15 m) and littoral Porphyra. Two levels of irradiance were used, one was full sunlight of approximately 12.5 $mWcm^{-2}$. The other was a "green shade" produced by screening the incubation bottles with one layer of "Cinemoid" Light Green (No. 23) filter material having a maximum transmission between 470-550 nm. This cut down the irradiance to 15% of the full sunlight as well as producing a spectrum quite close to that of the 15 m habitat. Because it was feared that local overheating of algal thalli might be occurring in these surface incubation experiments and perhaps combining with the high

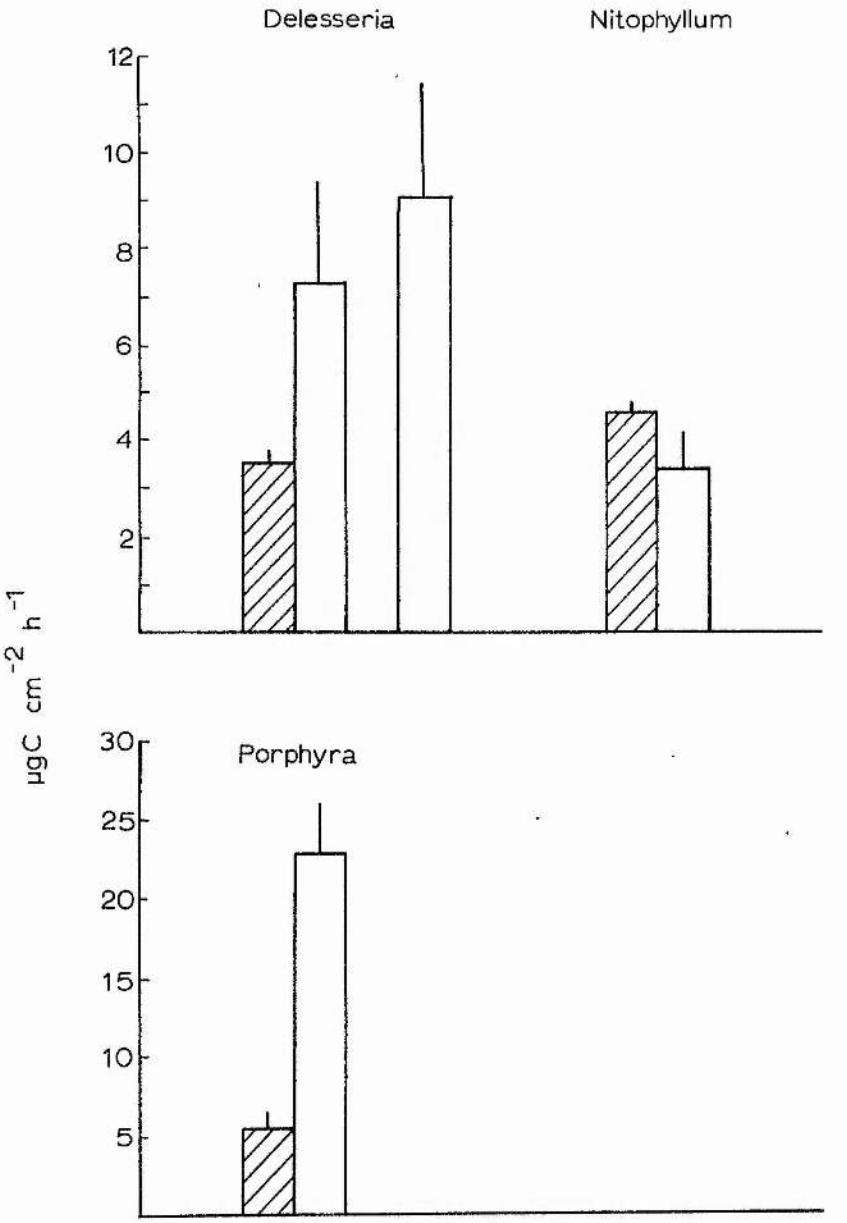


Figure 7.16. Rates of photosynthesis of three species at Durness at the surface in "green shade" conditions, ∿1.9 mW cm^{-2} (hatched columns) and full sunlight, ∿12.5mW cm^{-2} PAR (open columns); the third column for Delesseria represents tissue pretreated for 1h at 25°C; conducted in June; ^{14}C method; 14.5°C; 2h (1600 - 1800 BST). Photoinhibition evident in Nitophyllum.

irradiance in inducing photosynthetic inhibition, some specimens of Delesseria were pretreated at 25°C for one hour prior to incubation in the "full sunlight" treatment. The experiments were conducted at 14°C, compared with an ambient sea temperature of 9°C. Photosynthesis was measured by the ^{14}C method and the results are presented in Figure 7.16. Photoinhibition was positively shown in Nitophyllum only, where the photosynthetic rate in full sunlight was less than in the green shade. The photosynthesis of Delesseria in green shade was about 50% of that in the full sunlight. The mean rate of the 25°-pretreated material was in fact higher than in the untreated, but not significantly so, indicating no clear effect of temperature upon photoinhibition. Photosynthesis in the littoral Porphyra was greatly reduced in the green shade, to about 25% of the rate attained in full sunlight.

In the second experiment, specimens of Delesseria and Phycodrys (source 15 m) were pretreated by a one-hour exposure to sunlight of about 38 $mWcm^{-2}$ PAR with a covering of 5 cm seawater only (no glass). Control tissues were exposed concurrently in the same tank at the same temperature enclosed in black polythene to exclude sunlight. Samples were subsequently incubated, using the ^{14}C technique for 4 h at a mean irradiance of 25 $mWcm^{-2}$ PAR (maximum 35 $mWcm^{-2}$ PAR). At the end of the incubation period the pretreated material was observed to have lost most of its pigment, in both species, and appeared almost white. Carbon fixation rates are shown in Table 7.1

Table 7.1 Photosynthesis (^{14}C method) after pretreatment in either high irradiances (no glass cover) or in the dark (Temperature 14.5°C)

Species	Pretreatment (1 hour)	Incubation time (h)	Cumulative Irradiance J cm^{-2} PAR	Photosynthesis $\mu gCcm^{-2} h^{-1}$
Delesseria	38 $mWcm^{-2}$ PAR	3.6	495	0.02 ± 0.06
	Dark	3.6	360	2.17 ± 0.38
Phycodrys	38 $mWcm^{-2}$ PAR	3.6	495	-0.04 ± 0.03
	Dark	3.6	360	1.11 ± 0.03

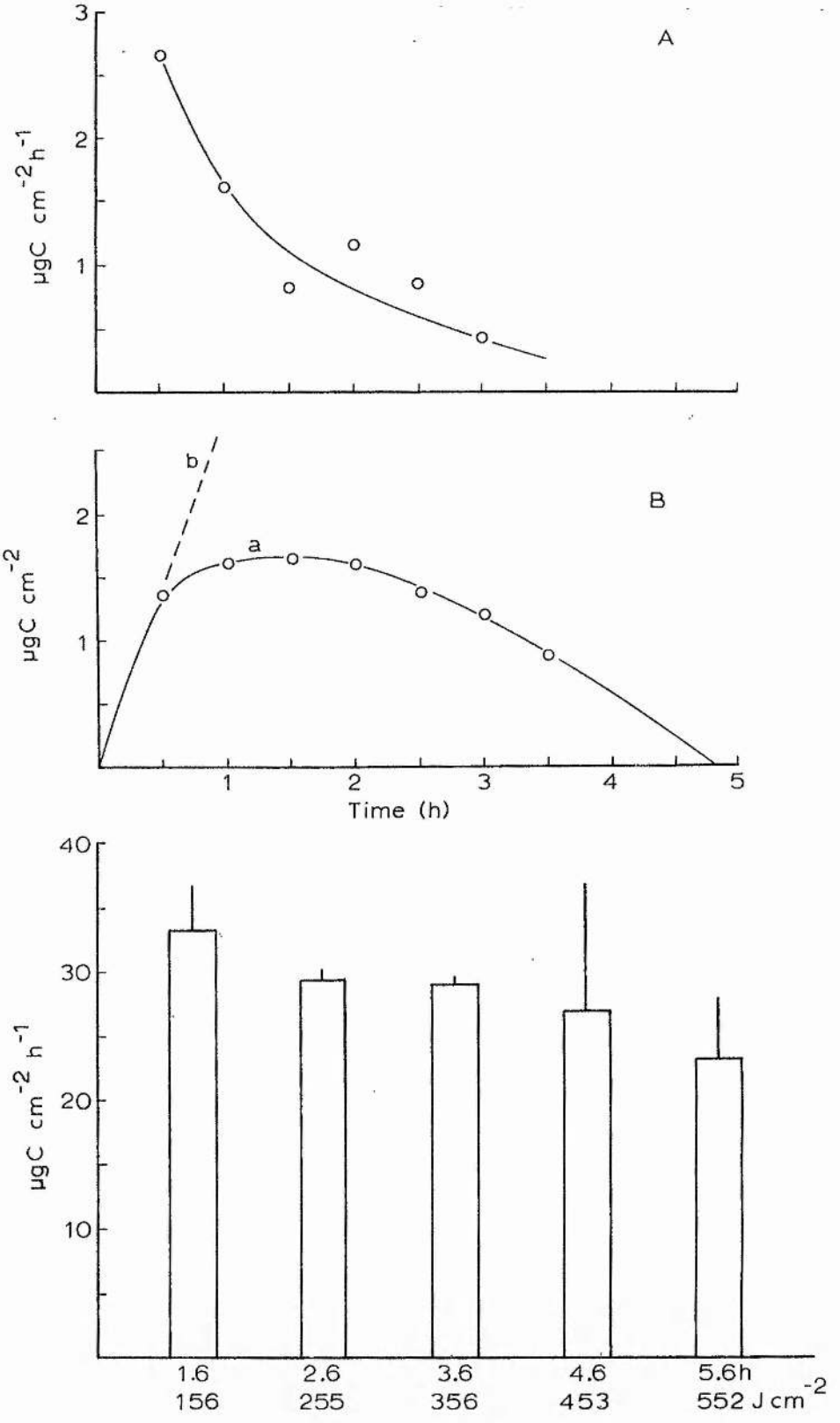


Figure 7.17. (upper). Photoinhibition of photosynthesis in Delesseria(source 18m) measured in surface sunlight, 22 mW cm^{-2} PAR, at Puffin Island A, Decline in carbon fixation rate measured at 0.5h intervals, curve fitted by eye; B, curve a, total carbon accumulated at end of each period of 0.5, 1.0, 1.5h, etc., showing loss of carbon after 1.5h (derived from curve in A); B, curve b, theoretical continuous increase in accumulation of carbon if photoinhibition did not occur. Experiment conducted in July; ^{14}C method; 19°C; 1330-1600h BST.

Figure 7.18. (lower). Photoinhibition of photosynthesis in Dilsea (source 9m, Fife Ness) measured in the laboratory at an irradiance of 28 mW cm^{-2} PAR for incubation times of 1.6 - 5.6h, giving cumulative radiation doses of 156-552 J cm^{-2} PAR; conducted in December; ^{14}C method; 12.5°C.

It can be seen that the pretreated tissue had carried out virtually no photosynthesis. In both species, one of the replicate samples, which had undergone pre-treatment actually had a lower ^{14}C activity than the dark control tissue (i.e. tissue pre-treated in sunlight and incubated in the dark) presumably indicating that the tissue was moribund. The results of this experiment can be compared with those shown in Figure 6.16 which show similar photoinhibition of photosynthesis in these two species.

Puffin Island site. Time-course of photoinhibition

The following experiment was carried out on Delesseria concurrently with an in situ and transfer experiment at 18 m and 3 m(see Figure 6.13C). Discs of material were cut from Delesseria freshly collected from 18 m depth, and placed in incubation bottles with ^{14}C bicarbonate and exposed, in a water bath at 19°C (sea temperature was 13°C), to full sunlight of mean irradiance 22 $mWcm^{-2}$ PAR (maximum about 25 $mWcm^{-2}$ PAR). At half-hourly intervals for a three hour period, individual bottles were removed and their incubation terminated. The carbon fixation rates of the discs, expressed per hour, are shown in Figure 7.17A. The rates decreased more or less continuously and a curve has been fitted to show the probable progress of photoinhibition. Values taken from this curve and plotted as the cumulative carbon uptake at the end of each half-hour period, produced curve (a) in Figure 7.17B. A curve of accumulated carbon fixation, assuming continuous photosynthesis of unchanging rate, would be in the form of a straight line passing through the origin (curve b, Figure 7.17B). It can be seen that fixation ceased altogether at 1.5 h and the carbon fixed in this time was lost over the remaining 1.5 h of incubation. It is suggested that this represents the pattern of events occurring in the second experiment at

Durness (Table 7.1) described above.

For comparison, the photosynthetic rate attained by 18 m Delesseria incubated in situ (see Table 6.4, Figure 6.13C) is shown, in Figure 7.17A to be higher than thehighest rate attained at the surface, in the present experiment, which was attained during the first half-hour.

Irradiation of Dilsea by the tungsten-iodide light source

Following the observation that slight photoinhibition appeared to occur when Dilsea was incubated at 28 $mWcm^{-2}$ PAR during the saturation experiment (Figure 7.5) the effect of pretreatment by exposure to high intensities of artifical light for longer periods was investigated. Discs of Dilsea freshly collected from Fife Ness, were placed in incubation bottles and exposed to full irradiance (28 $mWcm^{-2}$ PAR) from the tungsten-iodide light source for 4,3,2 and 1 h whilst shaking and keeping at 12.5°C. All bottles, plus two which had remained in the dark for 4 h, were then injected with ^{14}C bicarbonate and incubated under the same conditions for a further 1.5 h. The resulting carbon fixation rates are shown in Figure 7.18, together with the total "dose" received by each pair of replicates, expressed as Joules PAR. Great variability was exhibited by the tissue exposed for the longer two periods, but the mean values did decrease steadily from the maximum rate attained by the tissue which had received no pre-treatment in the light. The result indicates that some photoinhibition can be induced in Dilsea after prolonged exposure to high irradiance, but reference to Figure 6.13 for example shows that this species is tolerant of irradiances which are damaging to species such as Delesseria and Phycodrys.

5. Discussion

The photosynthesis-irradiance curves shown by the results of the experiments described above are very similar to those produced for marine algae by other authors (Monfort 1929; Kanwisher 1966; Mathieson & Burns 1971; Mathieson & Norall 1975; Fralick & Mathieson 1975; Kain et al. 1976). The curves exhibit all of the characteristics shown by the three basic curves shown in Figure 7.3. Tables 7.2 and 7.3 show I_k values, extrapolated by the method of Talling (1957) (see introduction of this chapter) for the data of several authors compared with those of the present study.

Table 7.2 A comparison of saturation irradiance levels and rates of photosynthesis (^{14}C method)

Species	Source depth(m)	Temp. °C	I_k mWcm^{-2} PAR	Photosynthesis µgCcm^{-2} h^{-1}	Author
Porphyra (Figure 7.4)		11	> 5	> 18.0	Present study
Rhodymenia (Figure 7.4)		11	3	32.0	" "
Delesseria (Figure 7.6)	9	11	1	11.9	" "
Dilsea (Figure 7.5)	9	11	2	22.5	" "
Odonthalia (Figure 7.4)	9	11	> 5	> 8.6	" "
Porphyra		10	10	30.0	Forbes (1975)
Rhodymenia		10	10	33.0	" "
Myriogramme		1	1	4.0	Drew (in press

Table 7.3 A comparison of saturation irradiance levels and rates of photosynthesis (oxygen method)

Species	Source depth(m)	Temp °C	I_k mWcm^{-2}PAR	Photosynthesis $\mu lO_2\ cm^{-2}\ h^{-1}$	Photosynthesis $\mu gCcm^{-2}\ h^{-1}$	Author
Rhodymenia (Figure 7.7)		18.5	2	11.7	6.3	Present study
Delesseria - Red (Figure 7.11)	6	16.0	1	4.8	2.6	" "
Delesseria Green (Figure 7.12)	6	16.0	> 5	>6.2	>3.3	" "
Polyneura (Figure 7.10)	18	18.5	0.5	4.8	2.6	" "
Polyneura (Figure 7.9)	18	18.3	0.5	5.5	2.9	" "
Polyneura (Figure 7.8)	4.5	19.3	1.4	1.2	0.6	" "
				$\mu lO_2\ mg^{-1}\ h^{-1}$		
Polysiphonia lanosa		10.0	2.7	11.4		Fralick & Mathieson (1975
Polysiphonia nigrescens	7	10.0	3.8	1.7		" " "
Chondrus crispus		15.0	2.7	3.3		Mathieson & Burns (1971)
Chondrus crispus		20.0	3.9	0.2		Kanwisher (1966
Phycodrys rubens	12	15.0	2.5	2.6		Mathieson & Norall (1975)
Eucheuma spp.	10-11	18.0	1.2-4.3	0.02		Mathieson & Dawes (1974)

It is seen that most species have an I_k value, that is saturation irradiance, around 2 mWcm^{-2} PAR. Thus, in a study of four sublittoral red algal species (including Phycodrys rubens), Mathieson & Norall (1975) found all species to be saturated around 2 mWcm^{-2} and all showed a decline, due to inhibition, above 5 mWcm^{-2}. Mathieson & Burns (1971) however, found that whereas the sublittoral

Chondrus crispus had a "shade-type" curve showing inhibition at 9 $mWcm^{-2}$, the similar Gigartina stellata from the littoral zone had a "sun-type" curve and showed no inhibition even at about 23 $mWcm^{-2}$. In a similar study on Chondrus crispus, but from the littoral zone, Kanwisher (1966) found no inhibition even at 23 $mWcm^{-2}$. Fralick & Mathieson (1975) found all three types of curve manifested by four species of Polysiphonia from intertidal and subtidal habitats but there was no constant correlation between shape of curve, and habitat. One littoral species and two sublittoral ones showed varying degrees of inhibition at 16 $mWcm^{-2}$, but one species showed none. Mathieson & Dawes (1974) found extreme variability in optimal irradiance for five species of the tropical massive red alga Eucheuma but found it to be in the range, 1.4 - 4 $mWcm^{-2}$, all species showing inhibition at the relatively low maximum irradiance level used, of 5.5 $mWcm^{-2}$. Drew (in press) found that of 13 species of Antarctic macroalgae studied, all were saturated at relatively low irradiances, ranging from 0.5 - 3 $mWcm^{-2}$, with no inhibition noted at the maximum irradiance of 15 $mWcm^{-2}$. In the present study, however, certain species, e.g. Porphyra and Odonthalia, Figure 7.4 and Delesseria (green thallus), Figure 7.12, did not exhibit clear-cut saturation points within the irradiance range employed, and probably reached saturation point above 5 or even 10 $mWcm^{-2}$ PAR. This result was found by Forbes (1975) for Porphyra using similar techniques (Table 7.2). Such high saturation values are characteristic of "sun" or high-irradiance-adapted plants, which, although this explanation may be valid in the case of Porphyra, seems unlikely in Odonthalia which was collected at 9 m and is typically a sublittoral species. The high I_k for Odonthalia may be due, not to a true physiological adaptation, but rather a physical characteristic viz. its high optical density which may give rise to an "inhomogeneity of light absorption" (Rabinowitch 1951, p.1007). It was postulated by Emerson & Green (1934) in experiments on the thick leathery-thallused, highly optically

dense Gigartina harveyana that, in a way analagous to the situation in dense cell suspensions, the photosynthesis of such species requires relatively higher irradiance to produce complete saturation because, as irradiance increases, light penetrates ever deeper into the thallus, continually bringing more cells up to saturation point. In thin algal thalli, and in dilute cell suspensions, saturation could be expected to occur with a sharper transition. Such an inhomogeneity of absorption could also be responsible for the gradual approaches to saturation shown by Rhodymenia (Figure 7.4) and Dilsea (Figure 7.5), both species with relatively optically dense thalli. This does not explain the situation in Delesseria with green thallus at Dunstaffnage (Figure 7.12) and in this case it seems fair to postulate that this ecotype, which is so visibly affected by its environment (the green thallus being produced by the destruction or "non-formation" of phycoerythrin due to high irradiance) is also physiologically adapted in the direction of "sun-adaptation" (i.e. Figure 7.3, curve a) perhaps correlated with a decrease in total pigment content (see Gabrielsen 1948). Such a raising of I_k in sun-adapted ecotypes may also be postulated as an explanation of the situation in Polyneura from 4.5 m and 18 m at Puffin Island (Table 7.3) and it will be noted from the tables that, with the exception of Odonthalia, lower I_k values are generally shown by the sublittoral species. Complex experiments involving growing of plants in shade and sun conditions and subsequently measuring their photosynthetic rates under different levels of irradiance have been conducted on higher plants by Bjorkman & Holmgren (1963) and Spence & Chrystal (1970a, b) and on unicells by Yentsch & Lee (1966) and Steemann-Nielsen (reviewed 1974). Such experiments have revealed that plants will adapt to the irradiance under which they are grown and so

produce sun or shade type photosynthesis - irradiance curves accordingly. Gabrielsen (1948) characterised both wavelength of incident irradiance and pigment content as "low-light" factors, and temperature and inorganic carbon source availability as "high light" factors. To this latter can be added enzyme activity, influencing the rates of the dark reactions of photosynthesis (Rabinowitch 1945, p.172). In this connection, Steemann-Nielsen (1976) has distinguished two main types of adaptation to different irradiance levels in phytoplankton. In one, the "Chlorella type", algae grown at low irradiance possess greater concentrations of chlorophyll than algae grown at high irradiance, resulting in greater efficiencies at the lower levels. In the other type, the "Cyclotella type", in algae grown at high irradiances, a rise in the saturation photosynthetic rate is noted compared with low irradiance-grown specimens and this has been attributed to an increased enzyme content in the high-irradiance algae, leading to a faster turnover in the dark reaction steps of photosynthesis (Steemann-Nielsen 1974, 1976). It is probable that these forms of adaptation occur in the macroalgae also, but to what extent and with what degree of mutual exclusivity is not at present known. Jupp (1972) found that deep specimens of Laminaria hyperborea had $\sim$ 8% more chlorophyll per unit area than shallow plants, but this was not accompanied by higher efficiences at low irradiances and thus did not appear to be a functional shade adaptation. From the curve shapes (Figures 7.11 and 12) it appears that the red and green forms of Delesseria represented shade and sun ecotypes respectively, both physiologically viable, and adapted to their particular irradiance microenvironment. Similarly, Porphyra and Rhodymenia (Figures 7.4 and 7) with their high saturation photosynthetic rates, are probably sun species, which may or may not possess the ability to develop shade characteristics under suitable conditions of low irradiance.

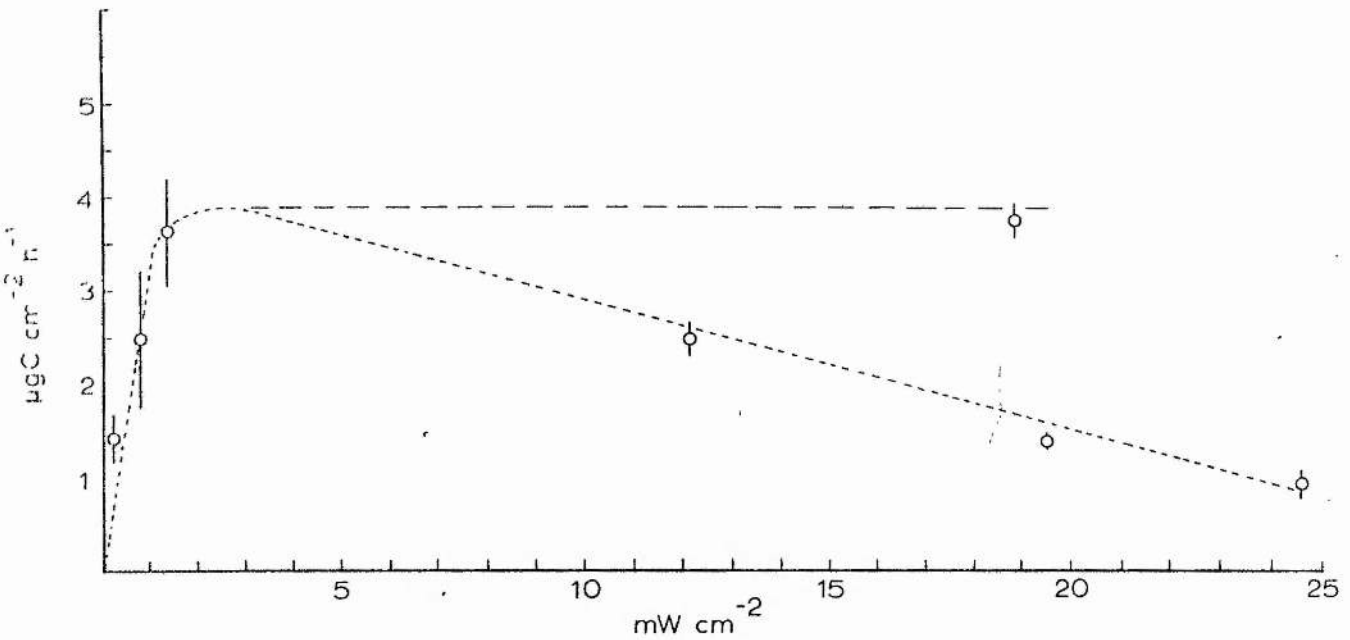


Figure 7.19 Rates of photosynthesis of Delesseria (source 18m) measured at various depths at Puffin Island (see Figure 6.13C) replotted with respect to measured irradiance; curve a, incubation time 4h; curve b, 1.4h. Experiment conducted in July, ^{14}C method, 13°C.

The photosynthesis-depth curves from the "transfer" experiments described in Chapter 6 are analagous to the photosynthesis-irradiance curves in this chapter except that in the former, the irradiance was reduced by a water column and this also continuously changed the spectrum of the irradiance (see Chapter 4). Photosynthesis-depth curves can be re-drawn in the form of photosynthesis-irradiance curves, if the in situ irradiance values are known, e.g. Figure 7.19, which is a re-plot of data from Figure 6.13C for Delesseria (source 18 m) at Puffin Island. The range of irradiance was produced by a combination of depth and variations in surface irradiance in the different experiments. It can be seen that the curve shape is similar to that in Figures 7.6 and 13, with an I_k of 1 - 1.5 $mWcm^{-2}$ PAR. There was no obvious indication that I_k occurred at a lower value as a result of the "green" spectral distribution of the irradiance as opposed to "white". Haxo & Blinks (1950) found that in Delesseria decipiens, saturation occurred at a lower irradiance in green (565 nm) than red (672 nm) light, in accordance with the action spectrum of this species. However, in the sea, irradiance within the photic zone is not exclusively monochromatic green and so enhancement by irradiance of other wavelengths may circumvent this effect. In absolute terms, the photosynthetic rate at saturation was lower in the in situ experiments than in the laboratory which may be due to the static conditions of the former.

It has been proposed (Montfort 1929; Rabinowitch 1951, p.995) that the photosynthesis-irradiance relationships of the green, brown and red algae typified the "sun", "intermediate" and "shade" curves, respectively, shown in Figure 7.3. From the results of the present study, this suggestion appears to be a great oversimplification, since all three categories are represented in the curves shown in Figures 7.4 - 13, i.e. all by species of

the Rhodophyta. Also, the chlorophyte Ulva, collected from 53 m at Ganzirri and transferred to shallower depths (Figure 6.7B) did show evidence of photoinhibition, a shade characteristic. It thus seems more reasonable to suggest that algae from the major divisions adapt to the irradiance levels ambient at their site of growth and do not exhibit adaptations which are entirely related to their taxonomic position. Thus, although in their detailed study utilising a sensitive oxygen electrode technique, Haxo & Blinks (1950) found that at irradiances less than 1 $mWcm^{-2}$ Delesseria decipiens reached saturation point much earlier than Ulva taeniata, this was correlated with shade and sun habitats respectively, rather than taxonomical position.

The initial slope of the photosynthesis-irradiance curve gives an indication of the maximum efficiency of photosynthesis (Gabrielsen 1948). There are many ways to define photosynthetic efficiency (as discussed by Phillipson 1966), one commonly used is the ratio, energy fixed:energy absorbed by the leaf (Gaastra 1958). In the absence of accurate knowledge of the effective absorption of radiation by the species concerned here, however, it is proposed to use the ratio, energy fixed:energy available to the radiation-absorbing plant organ (Gabrielsen 1948; Spence & Chrystal 1970b). In order to calculate photosynthetic efficiency, energy fixed and energy available must first be expressed relative to the plant character which limits or most strongly influences photosynthesis, in this case, lamina area (Sestak et al. 1971, p.21). They must also be expressed in the same energy units. The most suitable unit has hitherto been the gram-calorie, empirically derived from calorimetric studies of the specific heat of water, but the S.I. unit, the joule, is now favoured (Šesták et al. 1971, p.369), one joule being equivalent to 4.1855 gram-calories at 15°C. Throughout the present work,

irradiance has been expressed either in $Jcm^{-2}h^{-1}$ PAR or $mWcm^{-2}$ PAR, the latter being equivalent to 3.6 $Jcm^{-2}h^{-1}$ PAR. PAR has been used here as energy "available" to all species for photosynthesis, as a compromise between (a) total energy which includes IR which is scarecely utilised at all by plants, and (b) energy confined to wavelengths comprising specific absorption or action spectra of individual algal specimens. Photosynthesis measured either by the ^{14}C method, or converted from results obtained using the oxygen method (assuming P.Q. = 1) can be expressed as $\mu gCcm^{-2}h^{-1}$. (Photosynthetic efficiencies calculated from ^{14}C method results, or "gross" photosynthetic rates, will represent the maximum photochemical efficiencies attainable by the algae. Efficiencies calculated from rates obtained using the oxygen method will represent the "net" steady-state efficiencies of the algae and will be less than the former values. (In an ecological context, Phillipson 1966, prefers the "net" measurement.) It thus remains to obtain an equivalence between µg carbon fixed, and joules fixed. In respect of short-term experiments, it can be assumed that most of the carbon fixed is reduced, initially at least, to carbohydrate, in the red algae in the form of floridoside and mannoglycerate (Ceramiales), and floridean starch (Majak et al.1966) and in the green algae, as sucrose and starch (Meeuse 1962; Craigie et al.1966). The standard free energy change in the reduction of carbon dioxide to carbohydrate with the production of oxygen from water, is 502 J (120 k cal) per mole carbohydrate (Gregory 1971) which is equivalent to 4.1855×10^{-2} Joules fixed per µgC assimilated. Thus,

$$\text{Photosynthetic efficiency} = \frac{\text{energy fixed as carbohydrate}}{\text{energy available as PAR}} \times 100\%$$

$$= \frac{\mu gC \text{ fixed} \times 4.1855 \times 10^{-2}}{\text{J PAR available}} \times 100\%$$

$$= \mu g\ C\ J^{-1} \times 4.1855\ \%$$

Clearly, this computed relation makes many assumptions, but is of value at least in allowing a direct comparison of photosynthetic rates which have been measured at various irradiances in the limiting region of the photosynthesis-irradiance curve. As has already been stated, photosynthetic efficiency decreases exponentially with increased irradiance for values greater than I_k (Figure 7.2). The value of photosynthetic efficiency in making comparisons of photosynthesis at low irradiance has already been seen in Chapter 6 (e.g. Table 6.13) although the irradiances involved were perhaps not sufficiently low to be limiting. From Figure 7.3 it can be seen that, at low irradiances, "shade adapted" plants tend to have higher photosynthetic rates, and therefore higher efficiencies. This has been noted in studies comparing the photosynthetic activities of shade- and sun- adapted algal unicells (Yentsch & Lee 1966; Steemann-Nielsen 1974) in terrestrial plants (Gabrielsen 1948; Bjorkmann & Holmgren 1963) and in freshwater macrophytes (Spence & Chrystal 1970b).

Photosynthetic efficiencies calculated from the interpolated values of photosynthetic rate shown by the photosynthesis-irradiance curves for an irradiance of 0.5 $mWcm^{-2}$ (18 $J\ cm^{-2}\ h^{-1}$) are shown for gross photosynthesis measured by the ^{14}C method (from Figures 7.4-6) in Table 7.4 and for net photosynthesis as measured by the oxygen method (Figures 7.7-13) in Table 7.5.

Table 7.4 Photosynthetic efficiencies of algae measured at 1.8 $J\ cm^{-2}\ h^{-1}$ PAR (= 0.5 $mWcm^{-2}$) using ^{14}C method

Species	Source depth (m)	Photosynthesis $\mu gCcm^{-2}\ h^{-1}$	Efficiency %	From Figure
Porphyra	0	4.4	10.04	7.4
Rhodymenia	0	4.4	10.04	7.4
Delesseria	9	6.6	15.36	7.6
Dilsea	9	7.2	16.49	7.5
Odonthalia	9	1.6	3.73	7.4

Table 7.5 Photosynthetic efficiency of algae measured at 1.8 J $cm^{-2} h^{-1}$ (= 0.5 $mWcm^{-2}$) using oxygen method

Species	Source depth (m)	Photosynthesis $\mu lO_2 cm^{-2} h^{-1}$	Photosynthesis $\mu gCcm^{-2} h^{-1}$	Efficiency %	From Figure
Rhodymenia	0	5.25	2.82	6.57	7.7
Delesseria (red)	6	.28	0.15	0.35	7.11
Delesseria (green)	6	0.00	0.00	0.00	7.12
Polyneura	18	2.80	1.50	3.49	7.10
"	18	1.75	0.94	2.18	7.9
"	4.5	0.39	0.21	0.48	7.8

Considering Table 7.4 the efficiencies of the two sublittoral species Delesseria and Dilsea are higher than those of the littoral Porphyra and upper sublittoral Rhodymenia, and this fits with the hypothesis that the deep algae are shade adapted whilst the shallow algae are sun-adapted. These results are not in agreement with the finding in Chapter 6 that at low irradiances ambient at deep sites, shallow-grown algae tended to have efficiencies as high as or higher than the deep-grown plants (Tables 6.4 and 13). However, the irradiances encountered in the present study, at 18 m depth at Puffin Island, averaged around 0.9 $mWcm^{-2}$ PAR (3.4 J $cm^{-2} h^{-1}$ PAR) and were thus close to or above saturation point for most species, and, since shallow species tend to have higher saturation photosynthetic rates, then higher efficiencies would, inevitably, ensue in these species. The irradiances

measured at Puffin Island were probably absolute maxima, due to the ideal conditions, and since it is probable that the irradiance at 18 m during most of the growing season is in the range 0.2 - 2.0 $mWcm^{-2}$ PAR (calculated from the data of Kain et al.1975) it follows that the sublittoral species may in nature, habitually realise their maximal efficiencies. The very low photosynthetic efficiency of Odonthalia may be due to the extreme optical density of the thallus as already suggested and is not readily explained in terms of the sun-shade hypothesis.

In Table 7.5, considering first the different depth ecotypes of Polyneura, the deep-grown specimens are seen to have higher efficiencies than the shallow plants, consistent with the sun-shade hypothesis. In Delesseria, the red "unexposed" ecotypes had higher efficiencies than the green "exposed" ecotypes, again consistent with the sun-shade hypothesis. The very high efficiency of Rhodymenia, however, does not fit the sun-shade hypothesis. Since this was based on a net photosynthetic rate it may possibly be related to an extremely low respiration rate in the light, compared with other species (see Figure 7.7 and Chapter 8).

In absolute terms, the values for photosynthetic efficiency attained by the ^{14}C method (Table 7.4) are close to the range of 10.4-16.5% found by Gabrielsen (1948) for leaves of twelve species of tree (from gross photosynthetic rates, at 0.47 $mWcm^{-2}$ PAR) but greater than the value of 6% obtained by Spence & Chrystal (1970b) for freshwater macrophytes, using a manometric technique, which is more compatible with the results in Table 7.5 (net photosynthesis) obtained with the oxygen technique.

The high photosynthetic efficiences attained in the laboratory experiments are close to some of the highest ever reported, in the region of

20 - 30% for the unicellular alga Chlorella (Wassink 1959) and merit some consideration in terms of quantum efficiency, the ratio between molecules (or moles) of CO_2 reduced to quanta (or einsteins) of radiant energy absorbed by the pigment systems. Considering Delesseria (Table 7.4), 6.6 µgC were fixed for 1.8 J of available PAR. For the tungsten-iodide light source, a mean wavelength of PAR of ∿ 600 nm can be assumed (see Figure 2.11) and thus, from Figure 4.20,

$$1\ J \equiv 5\ \mu E$$

$$1.8\ J \equiv 9\ \mu E$$

By extrapolation from the absorption spectrum of Delesseria decipiens thallus (Haxo & Blinks 1950) and the spectral emittance characteristics of the tungsten-iodide source (Figure 2.11) it can be calculated that 35% of the quanta incident, between 300 and 700 nm are absorbed by the thallus, giving,

$$0.35 \times 9.0 = 3.15\ \mu E \text{ absorbed.}$$

Expressing the photosynthesis as moles carbon fixed,

$$\frac{6.6}{12} = 0.55\ \mu\text{moles carbon}$$

Since one einstein is a mole of a quanta,

$$\text{Quantum efficiency} = \frac{0.55}{3.15} = 0.175 \text{ moles einstein}^{-1},$$

which represents a quantum requirement of 6 quanta per molecule.

Since the minimum quantum requirement thermodynamically possible is 3-4 quanta per molecule (Gregory 1972) and the more probable figure for the two light reaction theory of photosynthesis is 8 (Heath 1969; Gregory 1972) we can

see that the efficiencies apparently attained in experiments using the ^{14}C technique are extremely high. Using similar ^{14}C methods, very high efficiencies have been noted in the Mediterranean green alga Udotea (100%) incubated at deep sites (Drew 1969) and in in situ experiments on L. hyperborea (38%, Jupp 1972). Considering the results from the oxygen method experiments, the highest photosynthetic rate, attained by Rhodymenia, was equivalent to 2.82 $\mu gC cm^{-2} h^{-1}$ at an irradiance of 1.8 J $cm^{-2} h^{-1}$ PAR, which, arbitrarily assuming the slightly higher effective absorption of 50% for this alga, and a mean wavelength in sunlight of 550 nm, a quantum requirement of 18 is obtained which is still quite low. Using an oxygen electrode technique, Yocum & Blinks (1954) found quantum requirements as low as 16 for Delesseria decipiens in green (500 - 560 nm) light. Since, in the present study, the oxygen technique measured net photosynthesis, the efficiencies tabulated in Table 7.5 would imply even higher values if gross carbon fixation were under consideration. Although accurate measurements of in situ irradiance were not made at Ganzirri, the estimates made were probably generous, giving Peyssonelia and Pseudolithophyllum efficiencies of 20 and 30% respectively (Table 6.2), suggesting that in the relatively "quantum-poor" irradiance of maximum wavelength 475 nm (Chapter 2), quantum requirements were 6 and 4 respectively, again close to the thermodynamic maximum. The highest efficiencies recorded in situ in Britain were 3.37% for Delesseria (Table 6.4) implying a quantum requirement of 26, and 7.77% for Dilsea below the canopy (Table 6.5) implying a quantum requirement of 15. It thus appears that macroalgae living at deep sites in the sea have the opportunity (due to the low ambient irradiance) and the capability to realise energy and quantum efficiences close to the maxima theoretically possible.

Briefly considering compensation point (dealt with more fully in Chapters 8 and 9), from the oxygen method experiments it can be seen that the

irradiance at which compensation occurred was below 1 mW in all species studied and this is within the range of 0.13-0.75 $mWcm^{-2}$ quoted for L.hyperborea (Luning 1971; Jupp 1972; Kain et al 1976), 0.04 $mWcm^{-2}$ for Plumaria elegans (Boney & Corner 1962) and, generally, for phytoplankton, 0.1-0.5 $mWcm^{-2}$ (Strickland 1958).

Considering, in absolute terms, the rates of photosynthesis attained by the algae in the present chapter, it has already been noted that the saturation rates attained in the laboratory experiments (Table 7.2) were much higher than those in the field experiments. There are two main considerations here, firstly, laboratory experiments utilised the ^{14}C method which is liable to yield results up to twice those from the oxygen method (Chapter 2; Drew pers. comm.) and secondly, laboratory experiments were shaken whereas field incubations were static, again involving a factor of about 2 (Chapter 3). Considering the rates attained by Rhodymenia then, we have 32 $\mu gCcm^{-2} h^{-1}$ attained in the laboratory, and 6.3 $\mu gCcm^{-2} h^{-1}$ (converted from 11.7 $\mu lO_2 cm^{-2} h^{-1}$) in the field, i.e. lower by a factor of approximately five, which could be taken account of by the combined factors described above. In comparing the results with those of other authors, collated in Tables 7.2 and 3, the results of the present study are of a similar order to those of others. Specifically the rate obtained by Forbes (1975) for Rhodymenia, using essentially the same methods as the present study, is very close to the rate for this species described here. However the disparity between Forbe's rate for Porphyra compared with that of the present work, and between the rates obtained for Chondrus crispus by Kanwisher (1966) and Mathieson & Burns (1971) call attention to the fact that, as in the in situ experiments, saturation photosynthetic rates for individual species can probably not be expressed more exactly than in terms of an order of magnitude, probably principally due

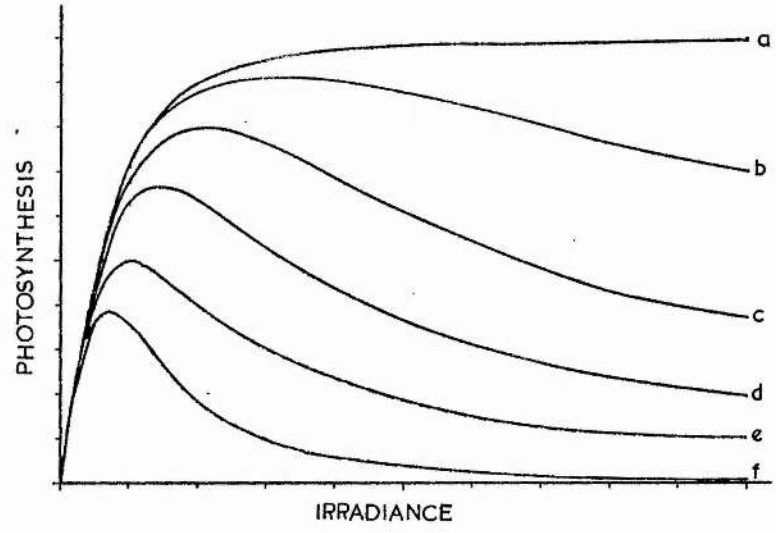


Figure 7.20. Possible progress of photoinhibition shown by photosynthesis-irradiance curves measured after exposure times which become progressively longer in the order a-f.

to the great variability of the plant material used.

Because of the frequent occurrence of photoinhibition of photosynthesis in the algae studied it is necessary to discuss the phenomenon in some detail. Also, because no adequate description exists in the literature to explain the process specifically with regard to red algae exposed to supra-optimal irradiance levels, a discussion will be given here of some of the possible mechanisms.

Photoinhibition is intricately connected with the pattern of light limitation and saturation as shown in the graphs in this chapter. Unlike limitation and saturation however, photoinhibition depends not only on level of irradiance, but also on time of exposure to it, as shown by Figures 7.18, Table 7.1 and certain of the in situ experiments described in Chapter 6 (Figures 6.9, 15 and 16). Determining the threshold conditions for the onset of photoinhibition is therefore not easy, since for example, a high irradiance acting for a very short period might not induce inhibition whereas acting for a much longer period it might do so. Considering the sublittoral species Delesseria, however, which was found to be relatively susceptible to photoinhibition, several experiments indicated that photoinhibitio did not occur around 20 Jcm^{-2} (5.6 $mWhcm^{-2}$) cumulative irradiance (Figure 7.6) but did occur at 40-50 Jcm^{-2} (11-14 $mWhcm^{-2}$) irradiance (Figure 7.6 and 18). It is probable that a series of saturation experiments conducted on such a species for different incubation periods might yield a family of curves as shown in Figure 7.20. Thus a relatively short incubation time experiment might not reveal photoinhibition at all (curve a), while successively longer incubation times might show a progressive increase in the photoinhibition effect (curves b, c, d, etc.). Such a progression of photoinhibition was recorded in Chlorella by Myers & Burr (1940). Bjorkman & Holmgren (1963) showed that although natural shade plants of Solidago had high saturation photosynthesis rates in initial short term experiments, a week of exposure to the relatively high irradiance of 15 $mWcm^{-2}$ lowered photosynthesis at

saturation to one third of its former value. In the present work, short term experiments indicate potential photosynthesis, and it must be kept in mind that for species like Delesseria, these high rates might decline with time.

The phenomenon of photoinhibition has been known to affect phytoplankton since early in situ studies (Marshall & Orr 1928). In laboratory experiments using solar radiation as the light source, Ryther (1956) noted extensive photoinhibiton in several phytoplankton taxonomic groups. Photoinhibition in macroalgae in natural sunlight was shown in the numerous field experiments of Printz (1939) and by Gail (1922), to occur principally in red algae transferred to shallow water from greater depths, e.g. Delesseria, Phycodrys, Odonthalia but also in the brown algal genus, Desmarestia. In studying the photoinhibition of Cladophora insignis, (green), Steemann-Nielsen (1952) found that the effects of one hour in an irradiance of 40 $mWcm^{-2}$ manifested themselves as a decline of photosynthetic rate which continued even after transfer to low irradiance (1.25 $mWcm^{-2}$). The damage was reversed after 24 h at this low irradiance, however. In the present study, occurrence of photoinhibition was confined to the sublittoral species and one species from the upper sublittoral (Rhodymenia), and bleaching of the inhibited specimens indicated that the effect was not reversible in the short-term (i.e. 24 h). There was evidence that shallow or "sun" grown ecotypes of Polyneura (Figure 7.8) and Delesseria (Figure 7.12) were less susceptible to photoinhibition than their deep or "shade" grown counterparts. Kanwisher (1966) specifically noted that he had found no photoinhibition of photosynthesis in Ceramium, Chondrus, Fucus, Ascophyllum, Ulva and Enteromorpha (all intertidal) in irradiances, from an artifical source, of up to 25 $mWcm^{-2}$ PAR, in comparison with the inhibition in phytoplankton noted by Ryther (1956) in sunlight.

Observations made in the present study suggested that plants which were exposed to bright sunlight in open containers, showed a greater degree of photoinhibition than specimens exposed in glass incubation bottles and/or under "Perspex" filter-holders or "Cinemoid" filters (Table 7.1 and Figure 7.18), and similar indications have arisen in studies of photoinhibition in L. hyperborea (Drew, pers.comm.). Because glass and the plastics involved are such effective UV filters (Chapter 2) the inference from these findings is that UV may be implicated in the mechanism of photoinhibition. Jitts et al.(1963) found no adverse effects of irradiance, from an artificial source, of up to 30 $mWcm^{-2}$ (400 nm - 650 nm) on the growth of several phytoplankton species, and attributed this finding (compared with the strong inhibitive effects noted by Ryther, 1956, in sunlight) to the lack of an inhibitive effect of UV which would normally be present in surface sunlight. Early work on the possible role of UV in photoinhibition revealed that although planktonic diatoms showed suppressed photosynthetic rates when incubated at depths from 0-10 m there was no significant difference between incubations carried out in normal glass containers (i.e. absorbing wavelengths <350 nm) and in "Uviol" UV-transmitting glass containers (Marshall & Orr 1928). However, Steemann-Nielsen (1964) in a study of the effects of UV on photosynthesis of phytoplankton, incubated shade-adapted ($\sim$1 $mWcm^{-2}$ PAR) cells in glass bottles (2 mm thickness) and either a neutral density filter (black silk cloth) reducing the noon sunlight by 70%, or this plus a glass sheet of 3 mm thickness. He found that the photosynthetic rate in the latter treatment was 20% higher than in the former conditions and concluded that UV was an important factor in photoinhibition manifested in deck measurements of photosynthesis in phytoplankton. In following-up laboratory studies of the lethal effects of far UV (254 nm, i.e. non-environmental) on Chlorella (Arnold 1934), Johnston & Levring (1946) found evidence of photosynthesis in near UV (366 nm) in several littoral macroalgae including Polysiphonia and

Enteromorpha, no inhibition being noted, although none might be expected at the extremely low irradiance used (0.035 $mWcm^{-2}$). Halldal (1967) has reviewed recent more sensitive studies of action spectra of algae in the UV. McLeod (1958) showed that photosynthetic activity of the unicell Porphyridium cruentum (Rhodophyta) was as great at 300 nm as at 440 nm and 680 nm (peak activity was ~550 nm, near the phycoerythrin absorption peak, as found by Haxo & Blinks, 1950). No inhibition was evident in the 1-2 h experiments in this species or the six other green, blue-green and diatom microalgal species studied, but the level of irradiance used was not specified. McLeod & Kanwisher (1962) found quantum requirements as low as 16 for Dunaliella (a dinoflagellate) at 350 nm and 450 nm, however a decline in quantum efficiency below 350 nm was tentatively ascribed to "screening" effects of absorption by non-photosynthetic proteins and amino acids (Green et al, 1974, give 512 nm as the upper limit of protein and amino acid absorption). Only at wavelengths of 290 nm and less did 15 min of irradiance at 5 x 10^{18} quanta (~2.3 mW) result in damage to the cells. Halldal (1964) produced action spectra for photosynthesis in the green Ulva lactuca (lower littoral) and red Trailliella intricata (gametophyte generation of Bonnemaisonia hamifera, 5 m depth). No oxygen evolution was recorded below 300 nm, but both algae showed high activity between 300 and 400 nm at energies around 0.5 $mWcm^{-2}$. Action spectra for photoinhibition showed a decrease from a maximum effect at 225 nm to a minimum at 310 nm beyond which no inhibition was induced at the energy levels (not specified) used.

Set against these laboratory photosynthesis studies are the considerable researches of Biebl (reviewed by Biebl 1959) on the effects of solar UV on cell vitality. Biebl (1952a) exposed specimens of littoral

and sublittoral macroalgal species to full noon sunlight at Plymouth at $\sim 44 mW cm^{-2}$ PAR (107 klux) and found all sublittoral plants, including Phycodrys and Polyneura to be bleached and dead after 2 h but all littoral species apparently unharmed. The same author (Biebl 1952b) found that on exposure to far-UV (230-310 nm) from a mercury vapour lamp (energies unspecified), Porphyra laciniata (upper sublittoral) survived 8 min irradiation whereas Ulva lactuca (upper littoral) survived only 1 min. Of 18 species tested, however, there was an apparent positive correlation between site on the shore-profile (including such sublittoral species as Delesseria sanguinea which survived only 1 min) and resistance to UV. Biebl (1952c) found consistently high absorption of UV (280-380 nm) in thalli of littoral species (Porphyra, Ulva) regardless of taxonomic division and low in sublittoral species (Phycodrys, Polyneura). However, these results did not give insight into the action spectra of UV since absorption could be taken as evidence either of sensitivity to, or protection (by "screening") from damaging effects. Biebl (1959) used glass filters to isolate UV (300-400 UV nm) and discovered that moribundity of cells in the surface of the thallus of the sublittoral red alga Calophyllis sp. occurred only after 13 times the dose normally required when full sunlight was used. Although showing that UV alone may not usually be responsible for the observed photoinhibition and photodestruction of algae, these experiments did show the relative potency of this very small proportion ($\sim 3\%$) of natural sunlight.

Although the bleaching and orange-fluorescence of red algae after exposure to bright light are well known to phycologists the mechanisms responsible are not well documented. The discolouration and destruction of light-absorbing substances in general, when irradiated in the presence of oxygen, is a well established event however (Egerton & Morgan 1970; Clayton 1970, 1971) known as photo-dynamism or photooxidation. Seliger &

McElroy (1965) have defined photodynamic action as "the photo-sensitised oxidation of the absorbate (or, frequently, a nearby substrate) of molecular oxygen". Photodynamism can thus be initiated by active absorption of radiation of any wavelength (UV, visible, IR) and is to some extent dependent upon relative photon energy. Franck & French (1941) observed an increase in oxygen uptake in the light, by Hydrangea leaves, and postulated that this was due to oxidation of photosynthetic intermediates, sensitised by chlorophyll, which was itself unaffected. Similarly, Steemann-Nielsen (1963) noted a depression of photosynthesis in shade-grown (3 klux ≃ 1.3 $mWcm^{-2}$) Chlorella when transferred to high irradiance (30 klux ≃ 13 $mWcm^{-2}$), but no damage to chlorophyll ensued. He explained this depression of photosynthesis, without any effect on chlorophyll concentration, as a "safety mechanism", postulating that excess energy absorbed by chlorophyll was actively dissipated via "back-reactions". (Photodynamic destruction and photosynthesis may be considered to be analagous processes, since the energy transfer mechanisms of both involve long-lived, light-excited states of the sensitising pigments; Spikes & Hall, 1963.) Sironval & Kandler (1958) studied bleaching of chlorophyll under high irradiance in Chlorella cells and found that chlorophyll concentration declined only after a time lapse or "induction period" and among other possibilities they postulated that perhaps only after all other available substrates had been photooxidised were pigments themselves destroyed. It seems possible therefore that photoinhibition of photosynthesis in red algae may be due to the photodynamic oxidation of phycoerythrin (and later chlorophyll) by chlorophyll and/or phycoerythrin itself occurring after the destruction of all other less vital reserves. In this activity UV may be more strongly implicated than the PAR wavelengths, due to the former's relatively high quantum energy (Chapter 4, Figure 4.20).

In considering the possibility that oxidisable substrates, such as storage materials, may offer protection from photooxidation of pigments, it will be recalled that the coarse thallused alga Dilsea, with a low SLA, showed

significantly less photoinhibition than certain more delicate forms with high SLA values, e.g. Delesseria and Phycodrys (see Figure 6.13). Similarly, Drew (1974b) found that photosynthesis in the delicate brown alga Desmarestia dresnayi from 18 m depth was reduced to zero, in full sunshine, after only 0.5 h, whereas this took 1.5 h to occur in the much coarser Laminaria hyperborea from the same depth. It may thus be that the large amounts of non-photosynthetic material in the thalli of the "coarser" species of algae acts as a photooxidisable reservoir, protecting the pigments from self destruction.

It has already been suggested (Chapter 2) that a non respiratory uptake of oxygen might occur in the light due to the reversible formation of epoxides from carotenoid pigments (Cholnoky 1956). It was first suggested by Griffiths et al. (1955) that the presence of carotenoids in photosynthesising plants might protect against photodynamic action since it was found that carotenoidless mutants of the photosynthetic bacterium Rhodopseudomonas perished when exposed to light in the presence of oxygen (but not in a pure nitrogen atmosphere) due to photooxidation of the protochlorophyll. It has since been established that epoxidation of zeaxanthin to antheraxanthin does occur in Euglena viridis (Krinsky 1968) and a "chemical buffer" role was proposed for this "carotenoid pair". In such reactions the protective function of the carotoids lies in quenching the excited triplet state of chlorophyll (Clayton 1964). In an ecological context, Calabrese (1972) and Calabrese & Felicini (1973) found that the carotenoid content in the Mediterranean red algae Petroglossum nicaeense and Gracilaria compressa was significantly higher in plants habitually receiving high levels of solar irradiance than in shaded plants or plant parts. The phycobilin content had the reverse distribution. This result points to the possible effectiveness of carotenoids in protecting the chlorophyll part of the

photochemical system, but their apparent failure to protect the phycobilins. Again, this may implicate the involvement of UV, since Krinsky (1968) has stated that the carotenoids offer no protection against ultraviolet irradiation.

In summary, there is evidence for the influence of UV wavelengths as well as high irradiances of PAR in the process of photoinhibition of photsynthesis. Both UV and PAR may initiate a photodynamic oxidation of substrates, including phycoerythrin, sensitised by chlorophyll. Carotenoids may protect sun-adapted plants by being reversibly oxidised photodynamically protecting other substrates.

CHAPTER 8

RESPIRATION

CONTENTS

1. Introduction

Respiration is the natural counterpart of photosynthesis whereby the energy and products fixed by the latter process are utilised and released again into the environment. Integral to any study of photosynthesis is the "compensation point" i.e. the point, measured in terms of some environmental factor such as light or carbon supply, at which carbon fixation by the plant just equals carbon loss, and on either side of which the plant makes net "profit" or "loss" accordingly. Since the development of the light-dark bottle technique of Gaardner & Gran (1927) and manometric techniques by Warburg, estimations of gross photosynthesis have been made on the assumption that carbon loss due to respiratory processes proceeded at a constant rate in dark and light conditions. It is now realised that in the light, completely separate respiratory processes occur, termed "photorespiration", which may supplement or replace the "dark" processes of oxygen uptake and carbon dioxide evolution which themselves may or may not continue unchanged in the light (see Heath 1969; Zelitch 1971; Gregory 1971; Tolbert 1974; p. of this thesis).

The respiration experiments described in this chapter must, therefore, be viewed in the light of this fact. They were conducted using the oxygen dark-light bottle method and are therefore measures of dark respiratory activity only. Their principal purpose was to monitor the levels of respiratory loss occurring in the species studied in the *in situ* photosynthesis experiments. The results will therefore be discussed in relation to

photosynthetic rates with reference to compensation points and photosynthesis: respiration ratios.

In certain cases, correlation coefficients have been calculated relating to scatter diagrams of respiration values with respect to temperature. Correlation coefficients are applicable only to linear relationships, and over the ecological temperature range (approximately 0-30°C), respiration v. temperature generally forms a "hump" shaped curve, with a maximum near the middle of the range and tailing off at both high and low extremes of temperature. However, over short ranges of 10°C or less, the relationship is probably close enough to linear to justify use of the coefficient.

2. Ganzirri

a. In situ experiments

At Ganzirri, the principal factor liable to influence respiration rate, and varying with depth, was ambient temeprature (p.132). With a knowledge of the direction of current flow in the Straits during an experiment, it was possible to decide whether the higher or lower of the temperatures recorded at the beginning and end of the incubation period had predominated throughout. Values of respiration rate plotted against temperatures calculated in this way, are presented as scatter diagrams for Ulva, Sphaerococcus and Laurencia in Figures 8.1, 3 and 6. Considering firstly Ulva, close scrutiny of Figure 8.1 shows that values for specimens from 4.5 m depth were generally higher than for material from 15, 30 and 53 m.

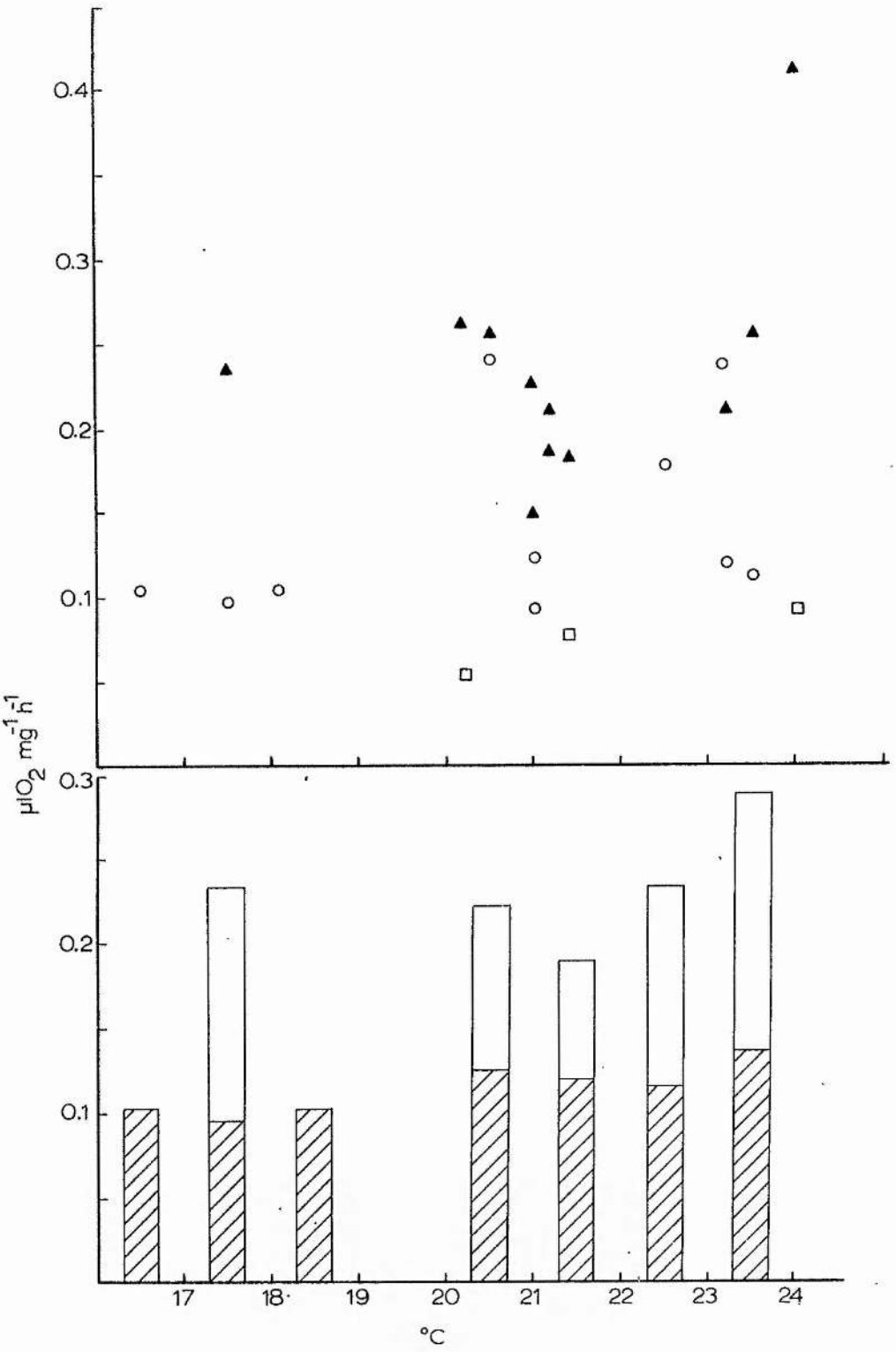


Figure 8.1. (upper). Respiration rates attained in situ by Ulva over the range of ambient temperatures at Ganzirri in September; tissue from; ▲ , 4.5m; □ , 33m; o ,53m.

Figure 8.2. (lower). Mean values plotted for single degree intervals from Figure 8.1; hatched portions indicate tissue from 33 and 53m, clear portions (total height), tissue from 4.5m.

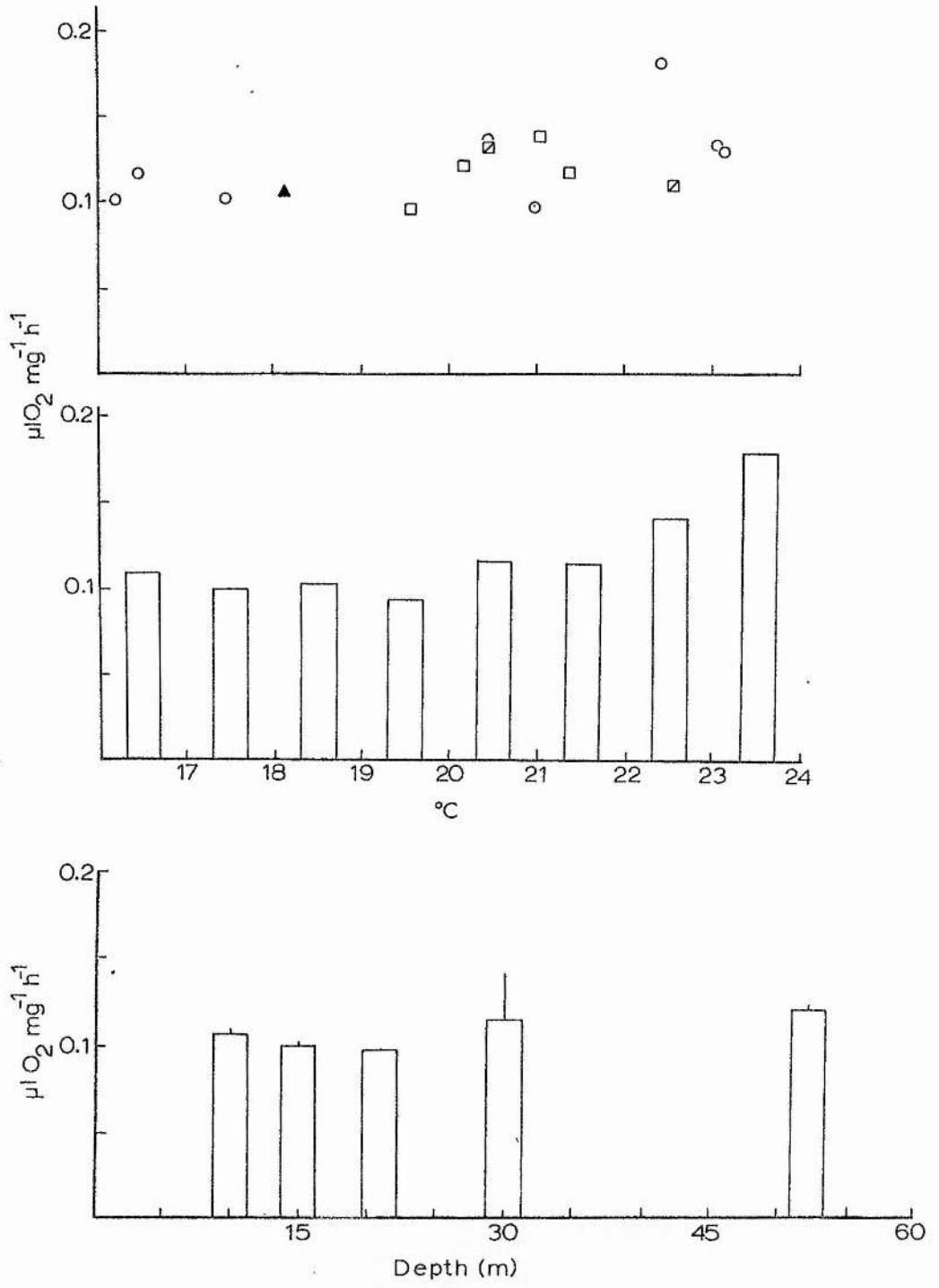


Figure 8.3. (upper). Respiration rates attained in situ by Sphaerococcus over the range of ambient temperatures at Ganzirri in September. Tissue from ▲ , 4.5m; ⊠ , 15m; □ , 33m, ○ ,53m.

Figure 8.4. (middle). Mean values plotted for single degree intervals from Figure 8.3.

Figure 8.5. (lower). Respiration rates attained by samples of Sphaerococcus collected from various depths and incubated under the same laboratory conditions at Ganzirri, September, 22.5°C.

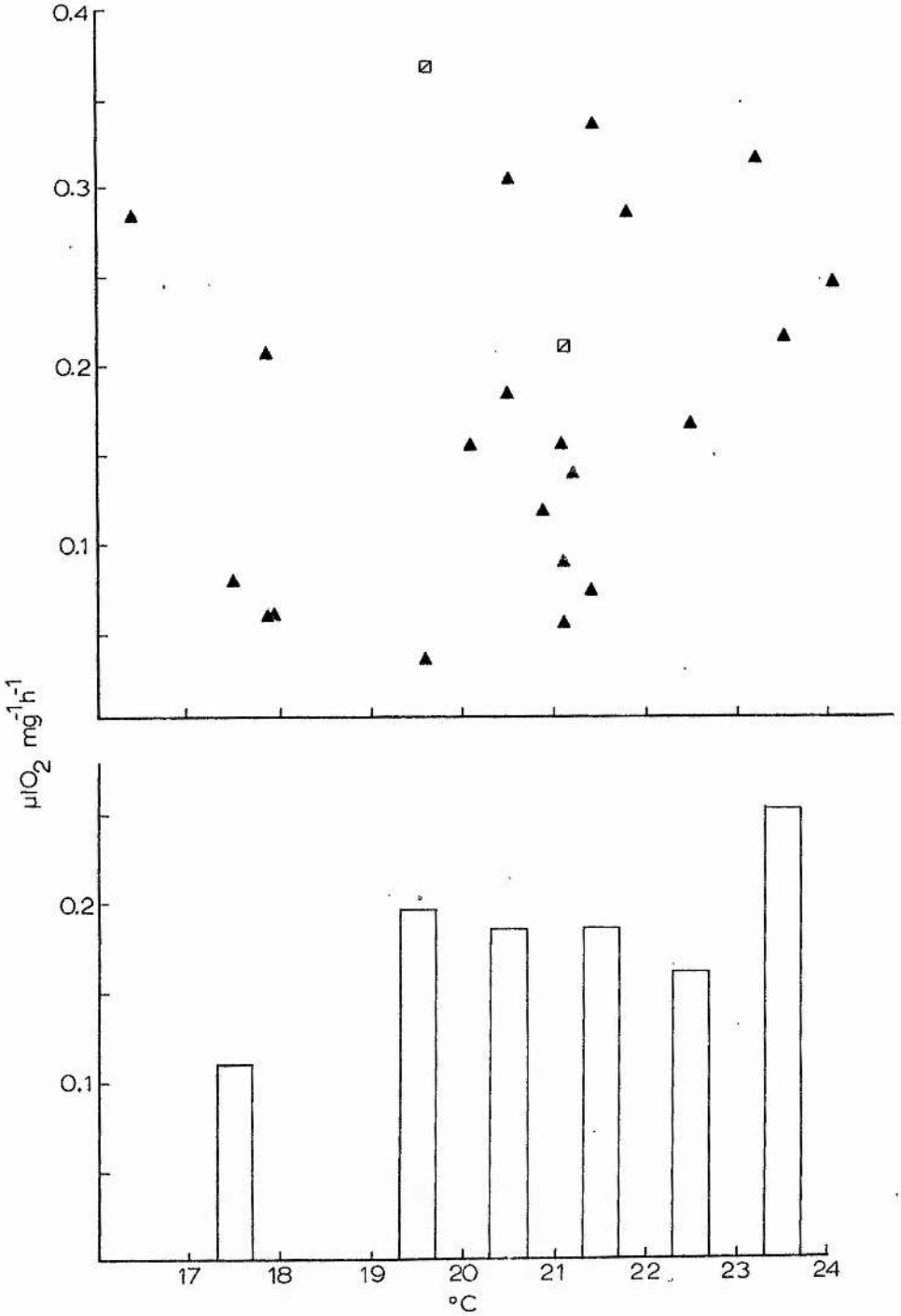


Figure 8.6. (upper). Respiration rates attained in situ by Laurencia over the range of ambient temperatures at Ganzirri in September. Tissue from; ▲ , 4.5m; ⊠ , 15m.

Figure 8.7. (lower). Mean values plotted for single degree intervals from Figure 8.6.

When the values are grouped into bands of single Centigrade degrees, a histogram constructed from the mean rate in each band (Figure 8.2) shows that the 4.5 m material (overall mean rate = 0.236 $\mu lO_2 mg^{-1} h^{-1}$, Table 8.1) consistently had rates which were approximately twice the values for the deeper specimens (overall mean = 0.126 $\mu lO_2 mg^{-1} h^{-1}$). The t-statistic calculated for the means for the shallow and deep specimens had a value of 4.24 which with 22 degrees of freedom, indicated that the means were significantly different at the 0.1% probability level. Respiration rates for both shallow and deep ecotypes were positively correlated with estimated temperature, but the correlation coefficients of 0.38 and 0.24 were not significant at the 5% level.

The rates attained by Sphaerococcus (Figure 8.3) showed less variability than the Ulva data, with a mean value of 0.131 $\mu lO_2 mg^{-1} h^{-1}$ (Table 8.1). There is no clear indication from Figure 8.3 that a consistent difference existed between respiration rates for the different depth ecotypes. The rates were positively correlated with the estimated temperature and the correlation coefficient of 0.51 was significant at the 5% level. The histogram in Figure 8.4, derived from Figure 8.3 shows the bias towards higher rates at higher temperatures. In a brief investigation of the degree of homogeneity of respiration of different depth ecotypes of Sphaerococcus, samples collected from 11, 15, 21, 30 and 53 m on the same day within a period of one hour, were incubated in temperature-controlled conditions at 22.5°C in the dark for 1.16 h. The respiration rates obtained presented in Figure 8.5, were fairly constant and though the 53 m material had the highest rates, these were only 1.2 times the lowest values, not the factor of 2 as found in the case of Ulva.

The results for Laurencia (Figure 8.6) were particularly variable (overall mean = 0.185 $\mu lO_2 mg^{-1} h^{-1}$) although all but two of the samples were collected at the same depth, 4.5 m. The correlation coefficient of 0.26

was positive, but not significant at the 5% level. The results are presented in histogram form in Figure 8.7.

Table 8.1 presents mean values of respiration rate obtained for all algae studied at Ganzirri in September and April.

Table 8.1. Respiration rates of Ganzirri algae

Species	Source depth (m)	Month	Temp °C	n	Respiration $\mu l O_2 mg^{-1} h^{-1}$	Respiration $\mu g C cm^{-2} h^{-1}$
Laurencia	4.5	Sept	20.7	22	0.185±0.021	
Gracilaria	4.5	Sept	20.0	3	0.150±0.039	
Pterocladia	4.5	Sept	20.5	2	0.372±0.094	
Vidalia	15	Sept	23.5	2	0.708±0.142	
Sphaerococcus	4.5-53	Sept	20.5	16	0.131±0.012	
Sphaerococcus	60	Apr	14.0	10	0.766±0.164	
Peyssonelia (calc)	53	Sept	23.2	2	0.234±0.067	1.751±0.299
(org)[a]	53	Sept	23.2	2	0.729±0.209	
(calc)	60	Apr	20.0	2	0.123±0.035	.968±0.279
(org)[a]	60	Apr	20.0	2	.385±0.110	
Pseudolithophyllum (calc)	53	Sept	16.5	2	.055±0.007	2.212±0.263
(org)[a]	53	Sept	16.5	2	.479±0.060	
Ulva	4.5	Sept	21.3	11	.236±0.016	0.165[b]
	53	Sept	21.0	13	.126±0.016	0.061[b]
	4.5	Apr	14.0	5	1.157±0.251	

[a] Assuming organic matter constitues 32% of calcified weight of Peyssonelia and 11.5% of calcified weight of Pseudolithophyllum

[b] converted using extracted SLA values of 0.767 for 4.5 m and 1.111 for 53 m material (see Table 5.6)

Considering the September data on a dry weight basis, the rate for Sphaerococcus was significantly lower than that for the shallow-growing species, Laurencia, but higher than the deep ecotype of Ulva, although not significantly so. The highest rate recorded was attained by the red Vidalia collected at 15 m. Peyssonelia had a relatively high rate on a dry (i.e. calcified) weight basis and on an organic matter basis had one of the highest rates. On a dry weight basis, the heavily calcified Pseudolithophyllum had the lowest rate recorded but on an organic matter basis this represented a relatively high rate. On an area basis, Pseudolithophyllum had the highest rate, and with Peyssonelia, both of these deep-growing red species had very much higher rates than deep or shallow ecotypes of the green alga Ulva.

In April, on a dry weight basis, the rates for Sphaerococcus and Ulva were approximately five times the September rates, but the variation was also substantially higher in April. Peyssonelia showed a reduction of about 50% from the September value. On an area basis, it is seen that due to the extremely high SLA (2.025 $cm^2 mg^{-1}$) of Ulva in April the respiration was in fact less than the corresponding rate in September. Since the April value for SLA of Peyssonelia was not measured its effect in modifying the reduction of rate in this month cannot be estimated.

b. Surface experiments

The relationship between respiration and a somewhat wider range of temperatures than available in the sea was further studied under more controlled conditions. The respiration rates of Sphaerococcus and Peyssonelia were investigated in April and of Sphaerococcus alone in September. A limited number of temperatures in the range 0-22°C was obtained by incubating

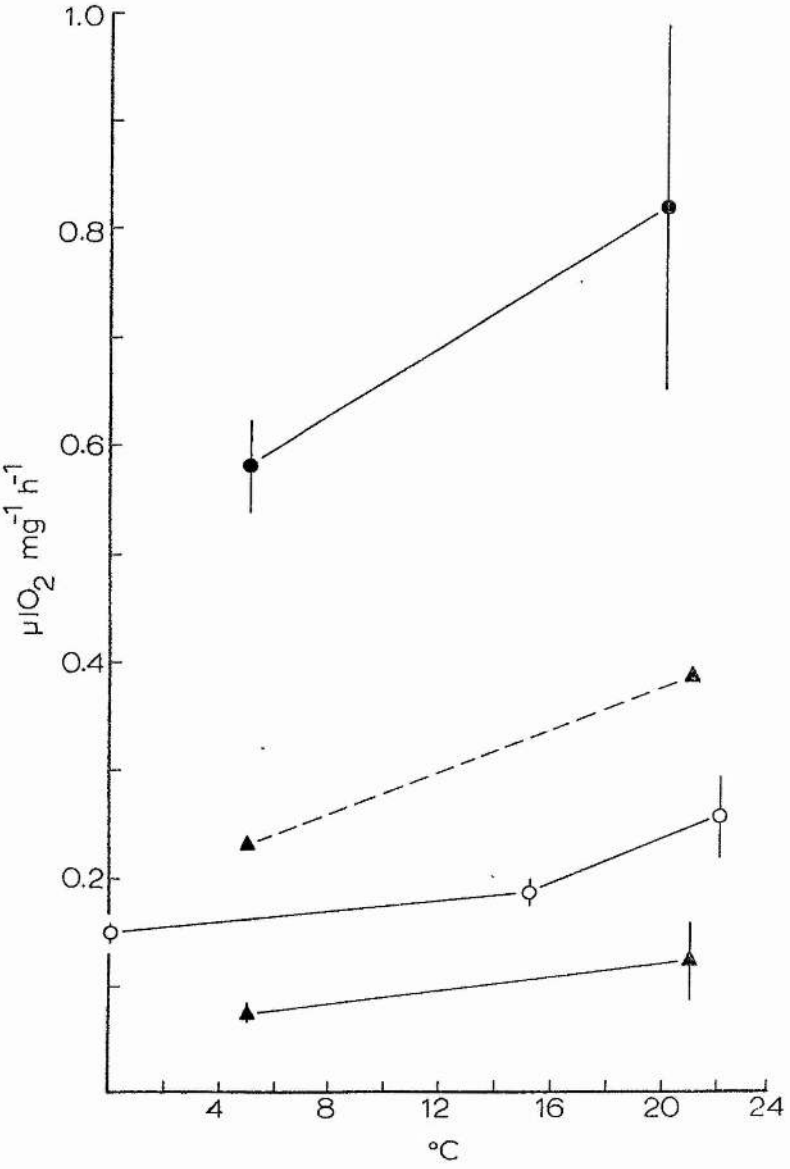


Figure 8.8. Relationship of respiration to temperature measured under controlled conditions at the surface at Ganzirri; O; Sphaerococcus in September; ● Sphaerococcus in April; ▲ , Peyssonelia in September; broken line represents results expressed on decalcified dry weight basis.

experimental bottles with tissue in a refrigerator, in aquarium tanks cooled by ice chips, and in tanks of seawater warmed by the sunlight. The results are presented in Figure 8.8. Firstly the disparity between absolute rates of Sphaerococcus in the different months should be noted. Both species showed a rise in respiration in relation to the rise in temperature in the range employed. As shown in Table 8.1, in absolute terms the rates for Peyssonelia are more comparable to those of Sphaerococcus on an organic matter basis than a dry weight basis. The temperature coefficient, Q_{10}, for the curves in Figure 8.8 have been calculated from the relation:

$$Q_{10} = \frac{\text{rate at } (t+10)^{\circ}C}{\text{rate at } t^{\circ}C} \quad ,$$

(Yemm 1965), for t = 0, 5, 10 and 15°C, and the values are presented in Table 8.2. In April, the Q_{10} values for Peyssonelia were higher than for Sphaerococcus, but in September the steep slope of the curve for Sphaerococcus between 15.5° and 23° yielded a maximum Q_{10} of 1.55. Over the range of 5-20°C, the Q_{10} values for Sphaerococcus were similar in April and September.

Table 8.2 Q_{10} values for Sphaerococcus and Peyssonelia

Temperature range °C	Sphaerococcus		Peyssonelia
	September	April	April
0 - 10	1.19	-	-
5 - 15	1.16	1.37	1.40
10 - 20	1.38	1.31	1.38
15 - 25	1.55	-	-

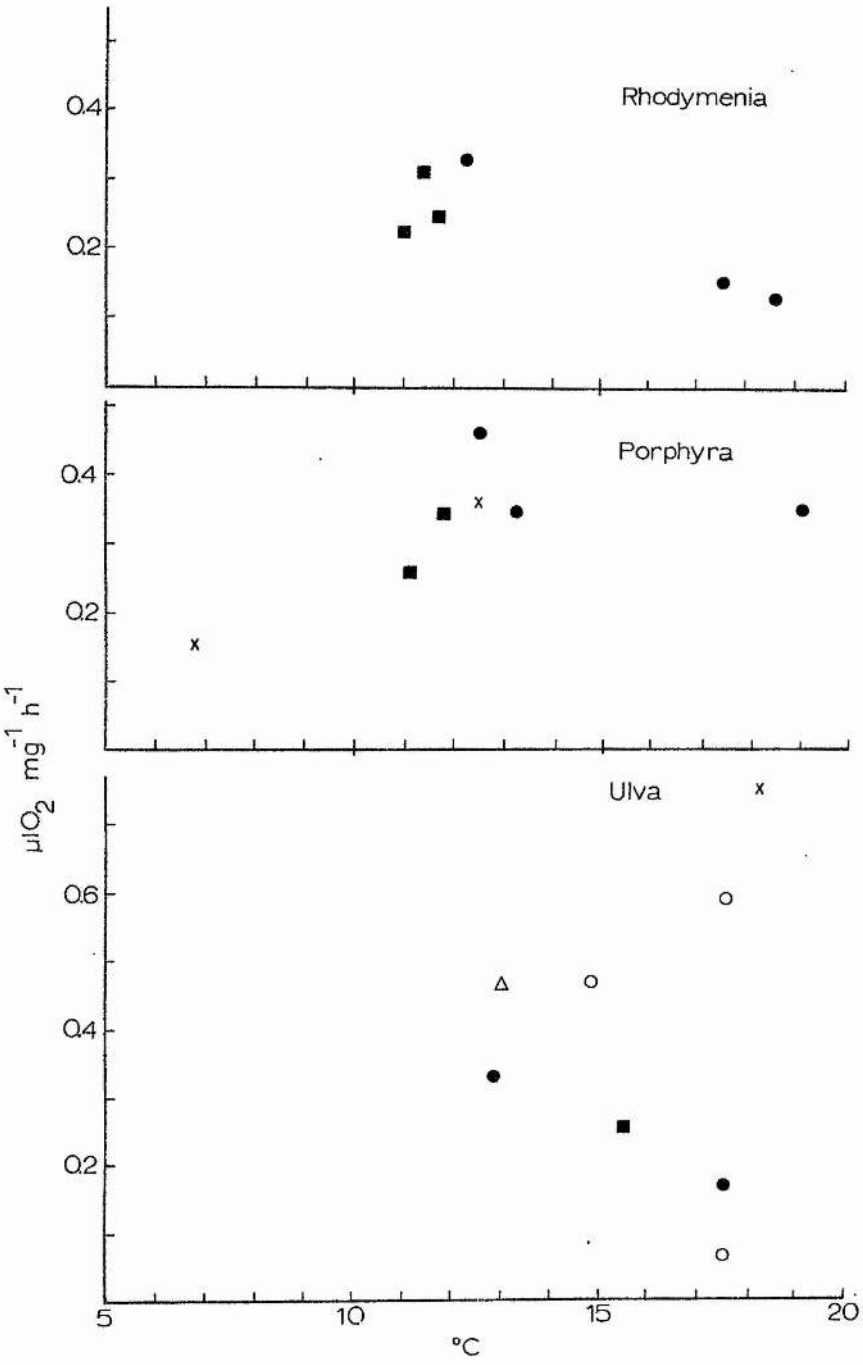


Figure 8.9. Respiration rates attained in situ by shallow algae at British sites; o , Puffin Island and Ballynablown; Δ , Dunstaffnage; □ , Durness; X, St. Andrews (in laboratory). Closed symbols - shallow material, open symbols - deep material.

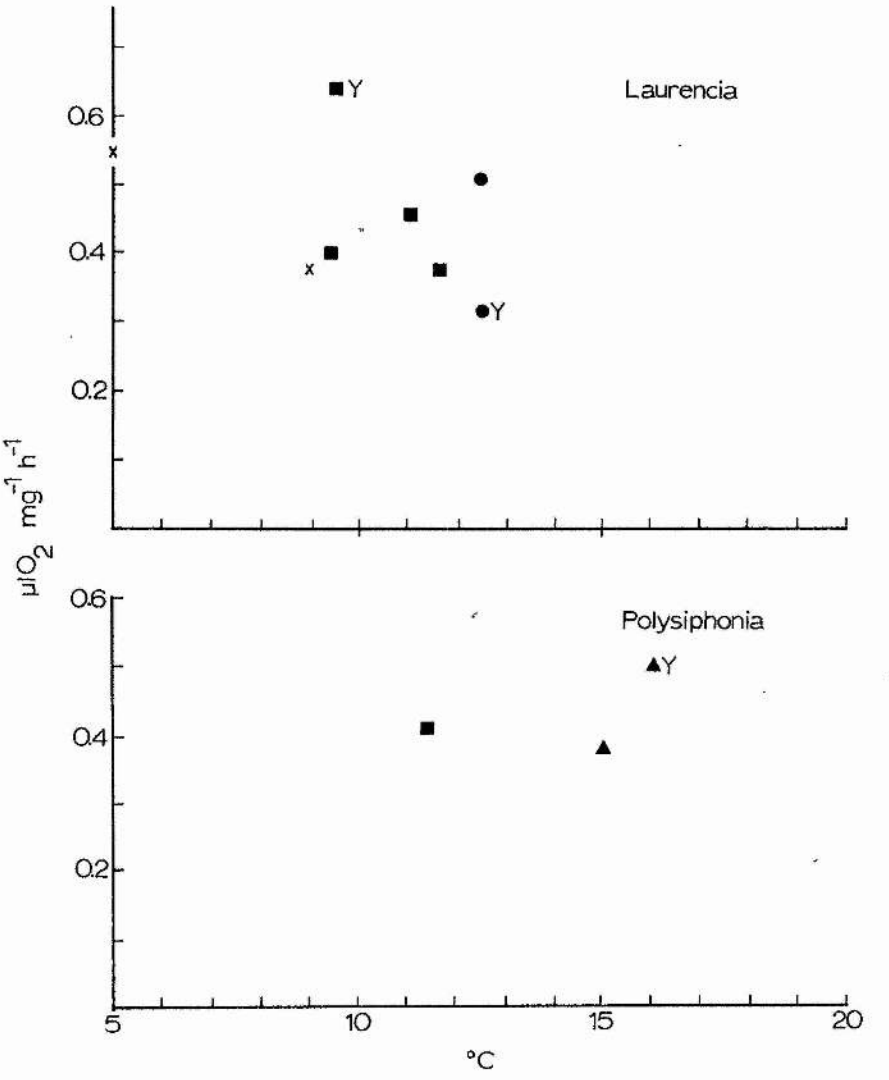


Figure 8.10. Respiration rates attained in situ by shallow algae at British sites; symbols as Figure 8.9.

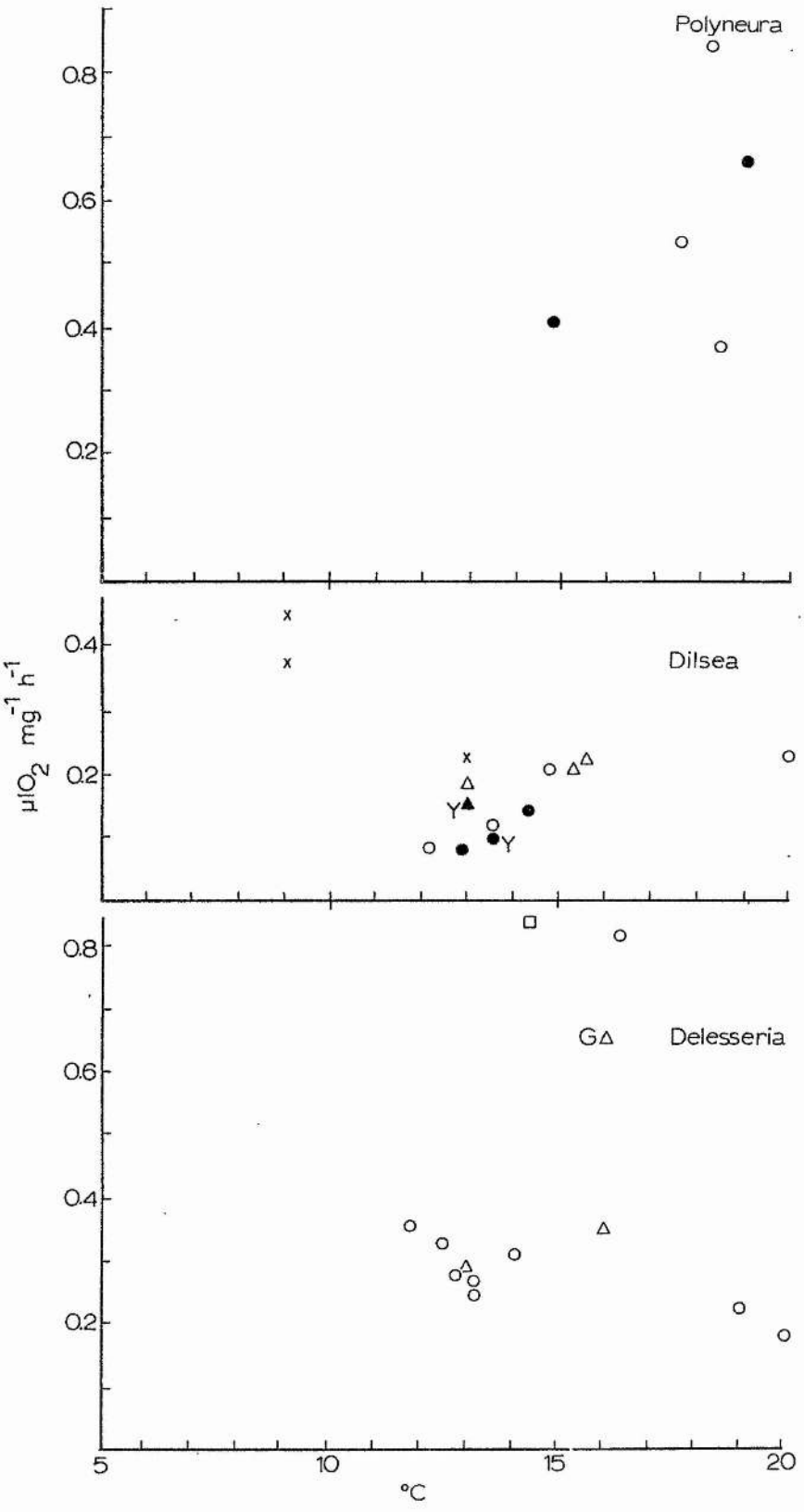


Figure 8.11. Respiration rates attained in situ by "deep" algae at British sites; symbols as in Figure 8.9.

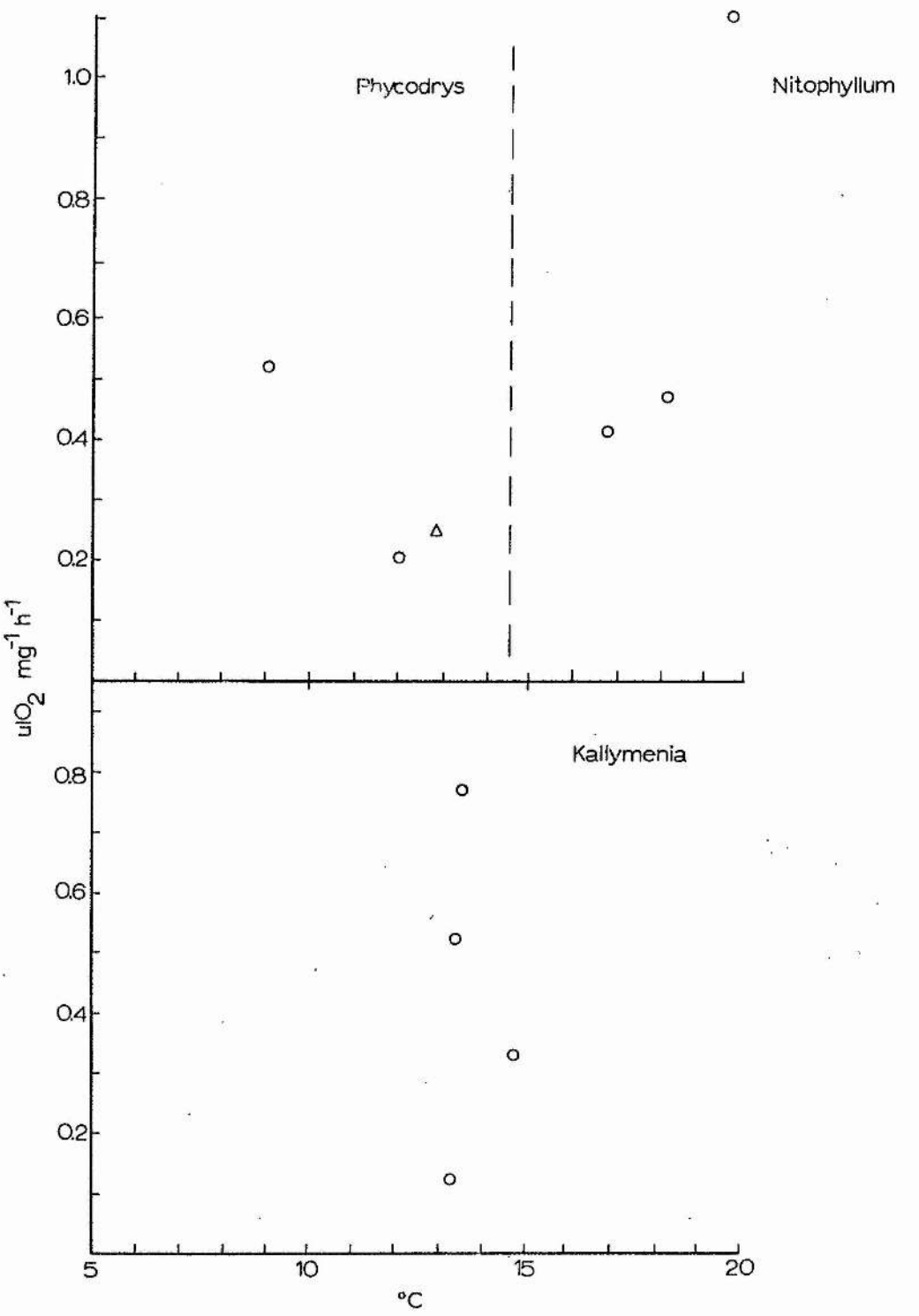


Figure 8.12. Respiration rates attained in situ by "deep" algae at British sites; symbols as Figure 8.9.

3. British Isles

a. Field experiments

Determinations were made either *in situ* concurrently with the photosynthesis experiments, i.e. at ambient sea temperature or in temperature-controlled conditions in tanks on the sea shore. Values of respiration so obtained are shown plotted against temperature of incubation in Figures 8.9-12.with an indication of month, location and site depth. In all cases where a sufficient number of determinations was made, the points exhibited a wide scatter. Correlation coefficients were positive in the cases of *P.umbilicalis* (Figure 8.9), *Dilsea* (Figure 8.11), *Polyneura* (Figure 8.11) and *Ulva*, source 4.5 m (Figure 8.9) and negative for *Delesseria* (source Figure 8.11), *Laurencia* (Figure 8.10), *Rhodymenia* (Figure 8.9) and *Ulva*, source 18 m (Figure 8.9). Only in the case of *Delesseria* was the coefficient of -0.66 significant at the 5% level, indicating a linear decrease in respiration with respect to temperature.

In order to permit comparison with the Ganzirri results and other data, a list of mean values was prepared (Table 8.3) summarising the values in the figures. However, since there is no reason to suggest that the different ecotypes in this study should have identical respiration rates, these means, derived as they are from results from a wide variety of sites and conditions, should only be considered as general indications of the rates attainable by the species concerned in the natural situation.

Table 8.3 Respiration rates of British algae, measured in situ

Species	Approx depth m	Approx temp °C	n	Respiration $\mu l O_2 mg^{-1} h^{-1}$	n	Respiration $\mu g C cm^{-2} h^{-1}$	SLA $cm^2 mg^{-1}$
Porphyra umbilicalis	0	14	5	0.352±0.032	3	0.721±0.058	0.262
P.leucosticta	1	13	1	0.343	1	0.299	0.538
Rhodymenia	1	13	6	0.234±0.034	3	0.495±0.081	0.254
Polysiphonia (red)	0	14	2	0.378±0.020	-	-	-
(yellow)	0	16	1	0.500	-	-	-
Laurencia (red)	0	10	4	0.302±0.035	-	-	-
(yellow)	0	13	2	0.482±0.161	-	-	-
Enteromorpha	0	12	1	0.773	1	0.927	0.448
Ulva	4.5	17	2	0.254±0.082	2	0.464±0.204	0.294
Ulva	18	16	5	0.370±0.093	5	0.469±0.103	0.424
Dilsea (red)	4.5	14	2	0.109±0.031	2	0.580±0.170	0.101
Dilsea (red)	18	14	7	0.180±0.020	7	0.958±0.096	0.101
Dilsea (yellow)	4.5	13	2	0.128±0.027	2	0.441±0.207	0.156
Delesseria (red)	6-18	15	11	0.419±0.069	10	0.627±0.093	0.359
(green)	6	16	1	0.555	1	2.060	0.101
Phycodrys	18	11	3	0.325±0.101	2	0.287±0.042	0.609
Polyneura	18	17	4	0.540±0.108	4	0.817±0.126	0.355
Polyneura	4.5	16	2	0.466±0.199	2	0.735±0.277	0.341
Nitophyllum	18	13	3	0.663±0.222	3	0.805±0.370	0.443
Kallymenia	18	14	4	0.439±0.137	4	0.510±0.174	0.463
Calophyllis	9	11	1	0.398	1	0.843	0.254
Plocamium	9	13	2	0.369±0.071			
Bonnemaisonia	18	13	1	0.589			

On a dry weight basis most of the rates were below 0.5 $\mu lO_2 mg^{-1} h^{-1}$. Considering the shallow species, the lowest rate was attained by Rhodymenia which was significantly lower than that of Porphyra, an alga of similar photosynthetic capacity (Chapters 6 and 7). The highest rate was attained by Enteromorpha (although this was based on only one pair of observations). Contrary to the finding at Ganzirri, Ulva from shallow sites had lower rates than specimens from deep sites, and both were low relative to Enteromorpha and most of the red algae. Of the deeper species, Dilsea from both 4.5 m and 18 m had substantially lower rates, of 0.109 and 0.180 $\mu lO_2 mg^{-1} h^{-1}$ respectively, than all the other species which showed values ranging from 0.325 $\mu lO_2 mg^{-1} h^{-1}$ (Phycodrys) to 0.663 $\mu lO_2 mg^{-1} h^{-1}$ (Nitophyllum). The value for 18 m Dilsea was greater than that for 4.5 m material although the latter value was based on two experiments only. Rates for deep and shallow material of Polyneura were not significantly different. In those species for which healthy but "bleached" material was available, viz. Dilsea, Delesseria, Laurencia and Polysiphonia, the bleached material had higher rates than the "normal" red tissue.

The rates for the two Porphyra species, very close on a dry weight basis, were widely different when considered on an area basis due to the difference in their SLA values. Alternatively, the rates for shallow and deep Ulva, quite separate on a dry weight basis, were very similar when considered on an area basis. In the deeper algae, however, by far the highest rate was attained by the green thallus form of Delesseria (2.06 $\mu gCcm^{-2} h^{-1}$) this being associated with the very low SLA for this species, of 0.101 $cm^2 mg^{-1}$. Similarly, the rates for Dilsea were much higher in relation to the other species than on a dry weight basis, the 18 m material having the second highest rate recorded, 0.958 $\mu gCcm^{-2} h^{-1}$, with an SLA for this thick-thallused species, of 0.101 $cm^2 mg^{-1}$.

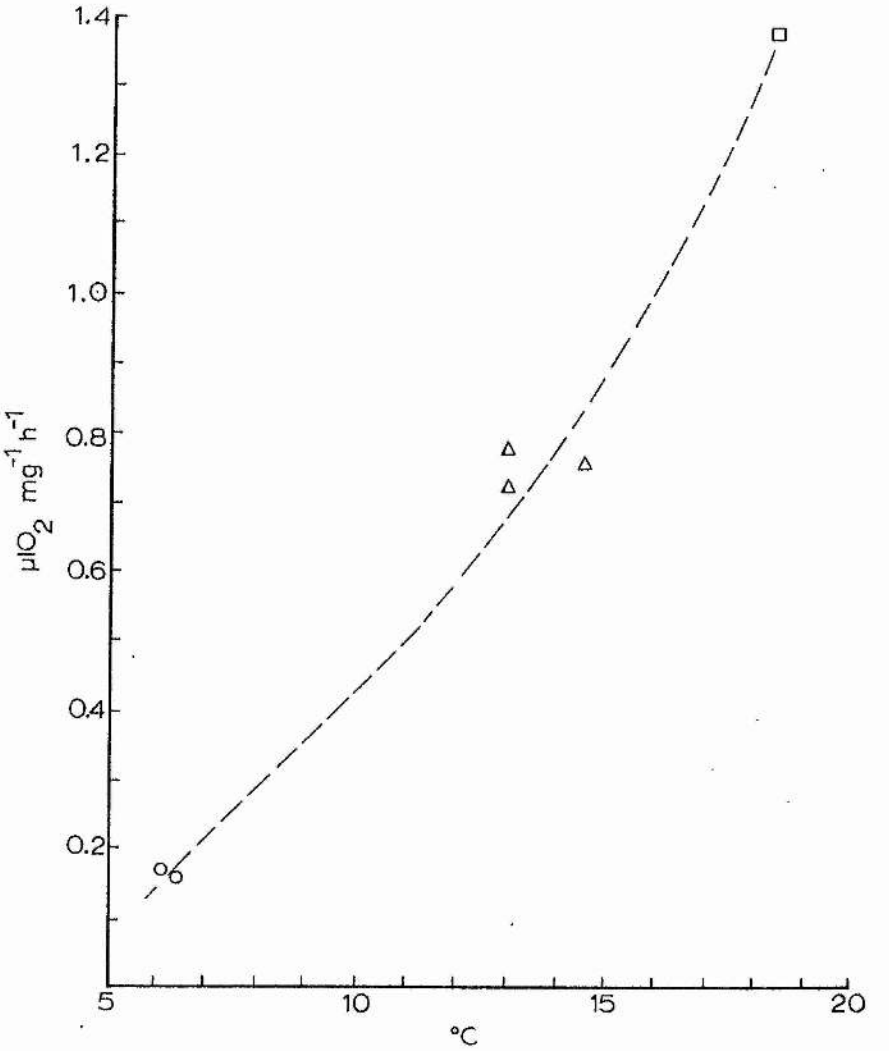


Figure 8.13. Respiration rates attained by Porphyra in the laboratory, with respect to temperature; o, December; Δ, June; □, July.

b. Laboratory experiments

Respiration rate measurements were conducted throughout the year, either in connection with photosynthesis experiments, or as part of investigations into methodology described in Chapter 3. Sublittoral species collected at Fife Ness and littoral species collected at St. Andrews were incubated in the constant temperature bath (p. 23), bottles being shaken or static according to the prime purpose of the experiment. Static experiments were considered to be more comparable with *in situ* incubations. The results are presented in Table 8.4. On a dry weight basis the results showed a considerable range of values but were generally higher than the *in situ* rates (Table 8.3). When plotted against temperature (Figure 8.13) it is seen that the range of values for *Porphyra* can be interpreted both as a progressive seasonal increase in rate, or as an increase in response to temperature. This curve gives the very high Q_{10} value of 10 for the temperature range 10-20°C. As in the *in situ* experiments, *Rhodymenia* had a lower rate than *Porphyra* at the same temperature. Also, as in the *in situs*, the rates for *Dilsea* were low compared with other species, and the June rates were higher than those attained in December. The rates for *Delesseria* and *Phycodrys* in May were extremely high, but due to low SLA values, were lower when considered on an area basis. As before, the rates for *Dilsea* on an area basis were very much higher than when considered in terms of dry weight. On a dry weight basis, the green alga *Ulva* had a lower rate than the red *Porphyra* under the same conditions, and computed on an area basis, *Ulva* had the lowest recorded rate.

Table 8.4 Respiration rates of British algae, measured in the laboratory

Species	Source depth (m)	Month	Temp °C	Shaken	n	Respiration $\mu l O_2 mg^{-1} h^{-1}$	Respiration $\mu g C cm^{-2} h^{-1}$	SLA $cm^2 mg^{-1}$
Porphyra	0	June	13.0	-	12	0.750±0.036	0.938[a]	
"	0	Dec	6.5	+	9	0.154±0.029	0.193	
"	0	Dec	7.0	-	10	0.142±0.017	0.178	
"	0	June	14.5	+	3	0.727±0.245	0.908	
"	0	June	13.P	+	14	0.708±0.029	0.885	
"	0	July	18.0	-	4	1.384±0.220	1.730	
Rhodymenia	1	June	12.5	+	2	0.378±0.077	0.820±0.091	0.248
Laurencia	0	Dec	9.0	-	4	0.375±0.013	0.879[b]	
"	0	Dec	5.0	-	2	0.556±0.191	1.303	
Dumontia	0	Dec	7.0	-	10	0.236±0.044		
"	0	Dec	7.0	-	10	0.207±0.016		
Dilsea	9	Dec	13.0	-	8	0.218±0.012	1.275±0.146	0.091
"	9	June	12.5	+	4	0.412±0.035	2.400±0.161	0.092
Delesseria	9	May	13.0	-	2	2.650±0.118	1.257±0.019	1.133
Phycodrys	9	May	13.0	-	2	1.761±0.196	0.732±0.019	1.293
Odonthalia	9	May	13.0	-	2	0.847±0.215	1.144±0.206	0.398
Ulva	0	July	18.0	-	4	0.729±0.035	0.683[c]	

[a] converted using SLA = 0.30 $cm^2 mg^{-1}$

[b] " " SLA = 0.16 $cm^2 mg^{-1}$

[c] " " SLA = 0.40 $cm^2 mg^{-1}$

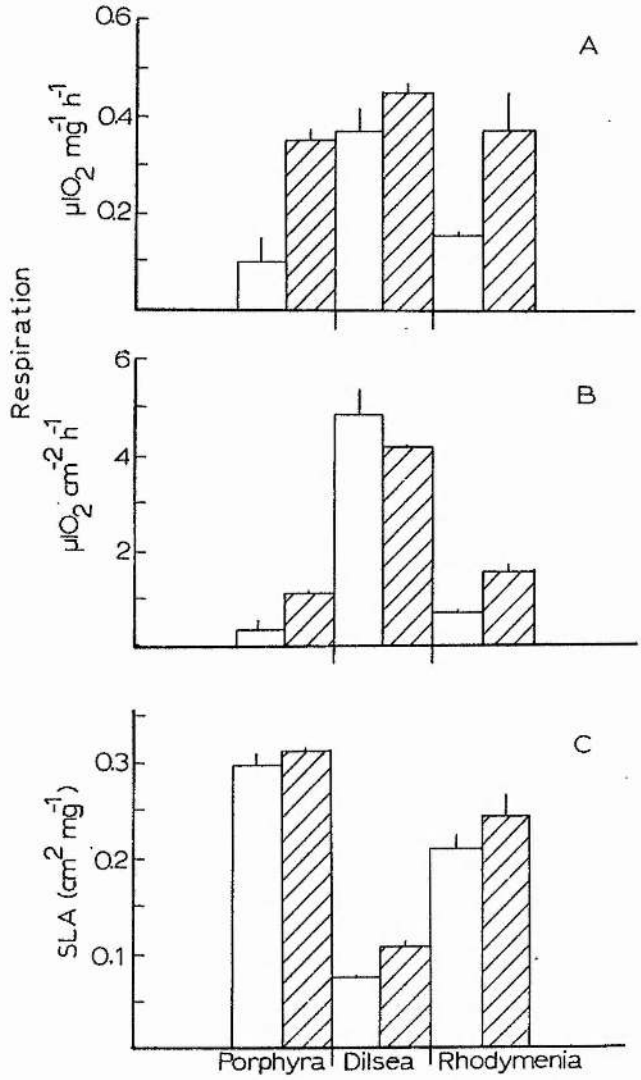


Figure 8.14. Effects of 14h pretreatment in light (clear columns) and dark (hatched columns); A, respiration, dry weight basis; B, respiration, area basis; C, SLA.

4. Effect of light and dark pretreatments on subsequent dark respiration

The finding (Chapter 3) that initial respiration rates in the early parts of incubation were considerably higher than the steady state rates produced speculation as to the effect of prior treatment on measured respiratory rate of the algae. Such effects would probably have more importance in laboratory experiments where an artificial (light or dark) situation frequently preceded incubation.

Specimens of Porphyra (intertidal), Rhodymenia (upper sublittoral) and Dilsea (9 m) were collected in the morning at low tide and transferred to a constant temperature room at 9°C. Half the specimens were kept in the dark and half were irradiated by two fluorescent tubes placed at a distance of 30 cm (approximately 1 $mWcm^{-2}$ PAR). The treatment was continued for 14 h after which time, discs of the various tissues were cut and incubated in the dark in the constant temperature bath at 12.5°C for 2 h. Respiration rates were measured, and the results expressed in terms of dry weight are presented in histogram form in Figure 8.14A. In all three species higher rates were consistently attained by the dark-pretreated material most notably in Porphyra where the rate was 3.5 times that in the light-pretreated tissue. Dilsea showed the least marked effect, and when calculated on an area basis (Figure 8.14B) the light-pretreated tissue actually had the higher rates, due to a differential in SLA values for the tissues in the two pretreatments. From Figure 8.14C, it is seen that in each species, the SLA of the dark pretreated material was higher than in the light pretreatment, and this had the effect that when expressed on an area basis, the differential in respiration rates between the two pretreatments was less than when expressed on a dry weight basis, since

rate per unit area α rate per unit mass $\div$ SLA.

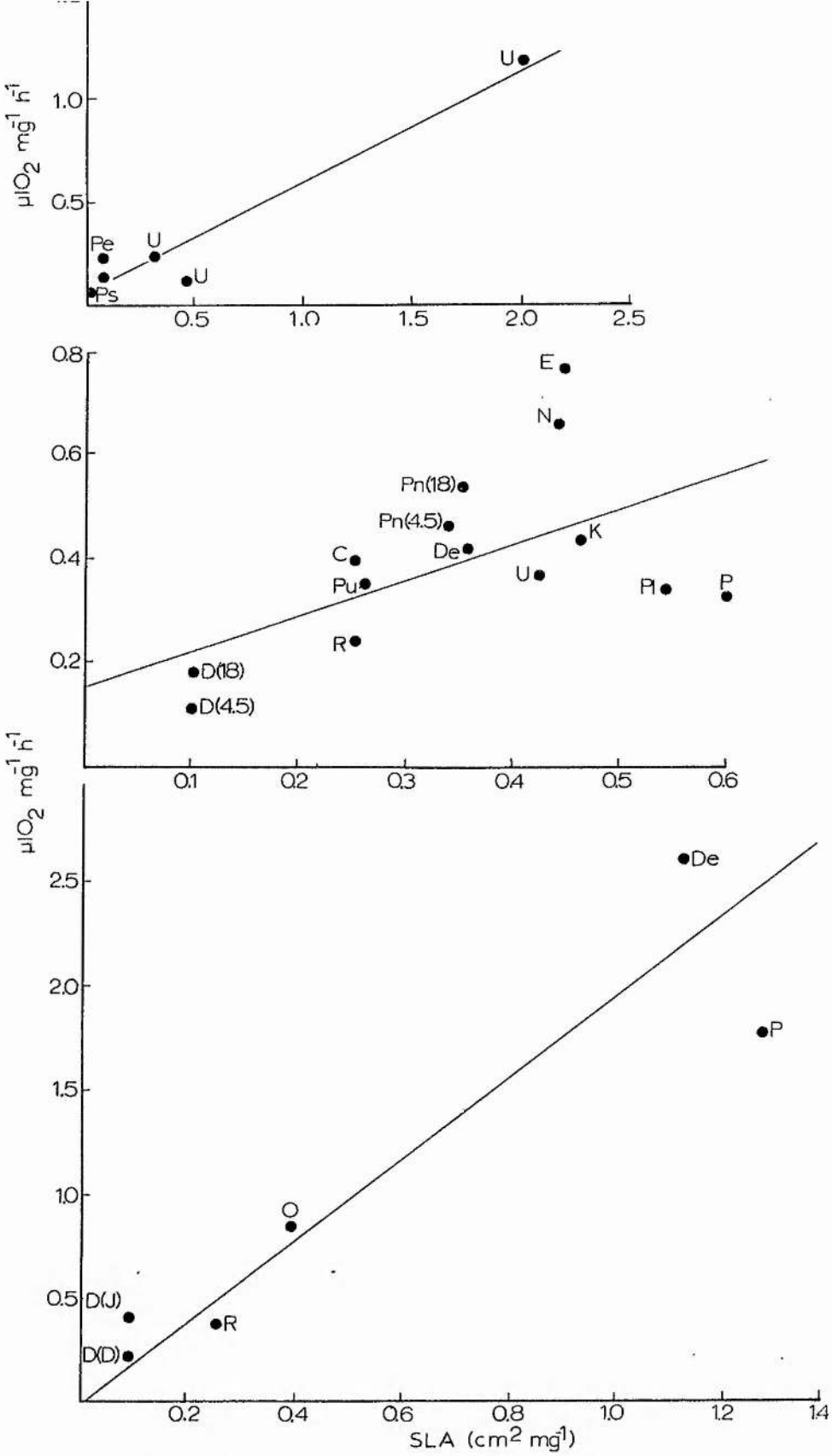


Figure 8.15. (upper). Fitted regression of respiration rate (dry weight basis) upon SLA in three species at Ganzirri; U, Ulva; Pe, Peyssonelia; Ps, Pseudolithophyllum(Table 8.1).

Figure 8.16. (middle). As above, for algae studied in the field in Britain; Pu, Porphyra umbilicalis; Pl, P.leucosticta; R, Rhodymenia; E, Enteromorpha; U, Ulva; D, Dilsea (sources 18 and 4.5m); De, Delesseria; P, Phycodrys; Pn, Polyneura (sources 18 and 4.5m); N, Nitophyllum; K, Kallymenia; C, Callophyllis (from Table 8.3.).

Figure 8.17. (lower). As above, for algae studied in the laboratory in Britain; symbols as for Figure 8.16 but including; O, Odonthalia; D (D) and D(J), Dilsea in December and June respectively Table 8.4).

5. Discussion

Before considering the respiration rates in absolute terms, the results merit consideration in terms of their relationships to the two reference bases used, namely dry weight and area. From Table 8.3 it was seen that the respiration rate of the thick-thallused (low SLA) Dilsea was lowest of all the algae studied, considered on a dry weight basis, whilst the rate of Enteromorpha, which has a high SLA, was the highest rate recorded. Figures 8.15, 16 and 17 are scatter diagrams for respiration rate ($\mu l O_2 mg^{-1} h^{-1}$) plotted against SLA ($cm^2 mg^{-1}$) derived from the data in Tables 8.1, 3 and 4 respectively. It will be recalled that low and high SLA values imply respectively thick and thin thallus forms, although this is not an exclusive rule. In both the Ganzirri data (Figure 8.15) and British laboratory experiments (Figure 8.17) there was a strong positive correlation between respiration and SLA both having correlation coefficients significant at the 1% level. In the in situ experiments in Britain, the correlation coefficient was again positive but significant at only the 5% level. (Delesseria with green thallus has been omitted from the calculations due to its anomalously high SLA for this species. It clearly did not follow the trend of the other results.) In each figure the regression line of y on x has been drawn showing the underlying relationships between respiration and SLA, and in the most significant cases (Figures 8.15, 17) the lines pass almost through the origin. Thus it appears that this process which is ultimately dependent upon oxygen uptake declines to zero when the area is zero (providing no surface for absorption) relative to mass. Put in simple terms, thick algae have lower respiration rates than fine, thin algal forms. This was substantially the finding of Kniep (1914, cited by Blinks, 1951) who, in a study of a range of red, green and brown seaweeds, noted that "coarse" algae such as Furcellaria

(red) and Fucus (brown) had lower respiration rates on a dry weight basis, than Enteromorpha, Ulva and Porphyra. Lüning (1971) working on Laminaria hyperborea and Clendenning (1971) on Macrocystis pyrifera found that fronds with lower dry weight per unit area (i.e. high SLA) had higher respiratory rates on a dry weight basis. Data obtained for L.hyperborea, L.saccharina and Saccorhiza polyschides however (Drew pers.comm.) did not show a statistically significant correlation between respiration rate and SLA. Variation of respiration in L.hyperborea fronds of varying "thickness" were reported by Kain et al.(1975). Why should tissue with a low SLA have a lower respiration rate? Lüning (1971) and Kanwisher (1966) suggested that such algae might contain a relatively higher proportion of "metabolically inactive" components (e.g. storage compounds). This situation was clearly shown in the two heavily calcified species at Ganzirri where the high proportion by weight of non-respiratory calcium carbonate resulted in a low rate for Peyssonelia and an extremely low rate for Pseudolithophyllum which is composed of 80% carbonate (Figure 8.15). It has also been suggested (Norton 1969 and Chapter 5 page 97) that thinner fronds may be an adaptation which increases oxygen availability to the internal tissues. It is thus tempting to suggest that internal tissues of thick thalli with low SLA had low respiration rates due to diffusion-controlled "oxygen starvation". Considering briefly an analogy in higher plants, Forward (1965) states that on the discovery that thin slices of tissue from bulky storage organs (e.g. Potato) had respiratory rates per gram which were much higher (5 - 10 times) than equal masses of the intact organ, it was generally assumed that respiration was less limited by diffusion of oxygen in the case of the slices. Subsequent research however, showed that an increase in oxygen concentration did not increase respiration correspondingly, and it was concluded that neither in the intact tuber, nor the slices, did diffusion

Table 8.5 Some laboratory determinations of respiration rate

Species	Site	Temp °C	Respiration $\mu l O_2 mg^{-1} h^{-1}$	Present study	Author
Porphyra (July)	Kent	15	1.43	1.38	Newell & Pye (1968)
" (Sept)	Kent	15	0.60		" "
Chondrus (July)	Woods Hole	15	0.18		Kanwisher (1966)
"		15	0.01		Mathieson & Burns (1971)
Polysiphonia	New Hampshire	15	0.01	0.38	Fralick & Mathieson (1975)
Polyneura	Vancouver	20	0.43	0.54	Brown & Tregunna (1967)
Vidalia	-	20	0.13	0.71	Gessner (1959)
Laurencia	-	20	0.49	0.19-0.56	"
Ulva	-	20	1.19	0.13-1.16	"
" (July)	Kent	15	0.45		Newell & Pye (1968)
" (Sept)	Kent	15	0.90		" "
"	Vancouver	20	1.05		Brown & Tregunna (1967)
" (July)	Woods Hole	15	1.00		Kanwisher (1966)
			$\mu gCcm^{-2} h^{-1}$		
Melobesioids	Eniwetok	29	6.76	2.20	Marsh (1970)
Peyssonelia	Malta	20	0.63	1.72	Drew (1969)

of oxygen limit respiration. The situation in the algae can only be elucidated by further studies of respiration in thin and thick (e.g. the massive tissues of laminarian stipes) algal material with reference to oxygen tension. The reduction of respiration rate per unit mass with increasing size of the organism is an established occurrence in the animal kingdom and is related to a reduction in surface-to-volume ratio. It had been suggested that heat losses (in plants as well as animals), facilitated by diffusive processes in "thin" organisms, might cause increases in metabolism to make up the losses, but since the phenomenon occurs in both cold-blooded (many marine) organisms and mammals, it is thought that factors other than heat loss must be involved (Zeuthen 1953; Odum 1956b). The converse of the relationships shown in Figures 8.15, 16 and 17 means that respiration per unit area decreases with increasing values of SLA, but the decreases will be exponential if the increases in the figures are linear, as suggested. Thus, Dilsea has a high rate on an area basis, 0.958 $\mu gC cm^{-2} h^{-1}$, whereas Phycodrys, with the highest SLA, has a rate of only 0.287 $\mu gC cm^{-2} h^{-1}$.

The increase of respiration on a dry weight basis, with increase in SLA is not without exceptions, notably the case of Ulva at Ganzirri (Table 8.1, Figure 8.15) where the deep material had a significantly lower respiration rate but a higher SLA than shallow plants. It has already been suggested (p.151) that these specimens may have belonged to two different species and this physiological difference may be a real one under genetic control. It is clearly of adaptive significance since low respiration rate, especially per unit area (Table 8.1) is important in lowering the effective compensation irradiance. It is also to be noted that, contrary to the majority of the findings reported above, Steemann-Nielsen (1976) has stated that the respiration rate of photosynthesising cells is generally

lower than that of cells unable to photosynthesise, due to the higher numbers of motochondria present in the latter type. In algae such as Dilsea, with low SLA, the proportion of non-photosynthetic cells is high, but respiration is low.

Considering the values of respiration rate in absolute terms, Table 8.5 shows that in many cases there is fairly close agreement with published results, e.g. Porphyra and Ulva (Newell & Pye 1968), Ulva and Polyneura (Brown & Tregunna 1967), Ulva (Kanwisher 1966), Laurencia and Ulva (Gessner 1959). Rates obtained by Fralick & Mathieson (1975) for Polysiphonia and by Mathieson & Burns (1971) for Chondrus were an order of magnitude below rates obtained in the present study and by other workers e.g., Kanwisher (1966). The rate for melobesioids (calcareous) obtained by Marsh (1970) was three times the value for Peyssonelia in the present work but was measured at a much higher temperature. Drew's (1969) value for Peyssonelia at Malta was substantially lower than that found in the present study. Wide disparity between estimates of respiration rate made by different workers for the same species, L.hyperborea, have been noted by Kain et al. (1976). Much of the variation in respiration measurements may be due to unrecorded differences in the physiological state and prior treatment history of the algae used in different studies (Levring 1947; Haxo & Blinks 1950; Kanwisher 1966; Šesták et al. 1971, p. 58).

Comparing the in situ rates for the Mediterranean with the British algae the mean rate attained at Ganzirri, of 0.354 ± 0.099 $\mu lO_2\,mg^{-1}\,g^{-1}$ (from Table 8.1) was not significantly different from the mean value attained in Britain of 0.399 ± 0.034 $\mu lO_2\,mg^{-1}\,h^{-1}$ (from Table 8.3). Specifically, the rates for Ulva at 4.5 m were not significantly different

in the two cases (considering September rates only in the Ganzirri case).

Seasonal changes in respiration were most evident at Ganzirri where Sphaerococcus and Ulva showed much higher rates in April and, in the latter species at least, this was correlated with an extremely high SLA value in this month (Table 8.1, Figure 8.15). Seasonal variations in respiration in British species were less obvious and were masked by temperature effects. Thus, Porphyra (Figure 8.9, Table 8.4) showed a general increase as the season progressed from December, through June to July, but this was also correlated with temperature. The Q_{10} of about 10 for this curve, however, is much higher than would be expected due to temperature alone (usually about 2), and may be correlated with a seasonal increase in SLA, noted elsewhere (Table 5.4) in this species. High rates for Delesseria and Phycodrys (Table 8.4) in May were correlated with high SLA values. Lüning (1971) (in common with Robertson 1970 and Jupp 1972) found that SLA of new frond material of L.hyperborea decreased from March to August and that respiration on a dry weight basis, measured at ambient sea temperature, varying in relation to SLA, reached a maximum in May and declined over summer to one third of this rate in August, thereafter declining gradually to December. In the same species, Kain et al.(1975) remarked that respiration of this species decreased, on a dry weight basis, as the summer proceeded, but since SLA decreased also, rate per unit area tended to remain constant. In six macroalgal species studied, including Enteromorpha, Ulva and Porphyra, Newell & Pye (1968) found that respiration (dry weight basis) decreased from July through September to December when measured at 15°C. In a study of six macroalgal species, including Enteromorpha and Ulva, Kanwisher (1966) found that respiration, measured at 20°C, was significantly higher (in all except Enteromorpha) in February and March than July and August. All of

these findings suggest that respiration reaches a maximum in spring, correlated with a high SLA, and declines during the summer in many cases this being correlated with a decrease in SLA which may itself be due to an increase in the content of storage materials which are metabolically inactive. However, there is evidence that intrinsic rates may increase also, since at Ganzirri, Ulva in April had three times the SLA in September, but five times the respiration rate, and in Britain, Delesseria and Phycodrys in May had respectively three and two times the SLA compared with later summer, but the respiration rates were six and five times as high in May.

Bleached thalli were found to have higher respiration rates than normal algae (and this was not correlated with increased SLA) in contrast to the findings of Hubbenet & Voblikova (1928) and Calabrese & Felicini (1973), who found that lower rates prevailed in specimens with reduced phycoerythrin. Since the bleached algae tend to have similar or lower photosynthetic rates (p.180) to normal, the extra respiratory load would appear to render them at a disadvantage compared with normal specimens.

Considering the influence of temperature on respiration, the rates of Ulva, Sphaerococcus and Laurencia at Ganzirri were shown to be positively correlated with the temperature of the seawater bathing the incubation bottles. That this correlation was detectable is somewhat remarkable in view of the assumptions made in the method of temperature estimation, and the fact that correlation in the case of at least one species (Sphaerococcus) was statistically significant suggests that the respiration rates of algae growing in the Straits fluctuates regularly as the current flows alternately north (cold) and south (warm). Figures 6.1, 2, 4, 5, 6 and 8 showed the respiration rates measured in situ in the Ganzirri experiments, and it can

be seen that in most cases, rates were lower at the deeper sites, in accordance with the ambient temperatures (see Figure 5.3). The Q_{10} values of 1.16 to 1.55 (Table 8.2) for the range 10-20°C are low for the process of dark respiration which generally has values from 2-4 in plants (Yemm 1965). The value of 1.4 obtained for Peyssonelia is lower than that of 2.3 found by Drew (1969) at Malta for this species over the same temperature range. Q_{10} values as low as 1.06 were recorded by Newell & Pye (1968) for certain British species however, and these workers found that the respiration-temperature curve was shallowest (i.e. low Q_{10}) in the temperature range appropriate to the ambient sea temperature. This suggests that the low Q_{10} values for the Ganzirri species may be an adaptation to the short-term fluctuations in the environmental temperature. In British algae, in the present study, there was no clear relationship with temperature, shown by the results of the in situ experiments, both positive and negative correlations being present (Figures 8.9-12). This suggests that variations due to season, site and biological differences may be of more significance than temperature. Only in the case of Delesseria (Figure 8.11) was the correlation significant, in this case negative, for a temperature range of 12-20°C, indicating that inhibition was occurring at higher temperatures. In temperate algae, temperature optima as high as 35°C have been reported for shallow green algae Enteromorpha and Ulva (Newell & Pye 1968) but equally, certain shallow algae, e.g. Porphyra showed a decline in rate above 25°C. The generally greater susceptibility of sublittoral algae, like Delesseria, to extremes, high and low, of temperature, is well known (Biebl 1962, 1972), and this may account for the negative correlation in Delesseria, although in this limited study, the rule of negative correlations in sublittoral species and positive in shallow species was by no means

universal. As with the action of irradiance in photoinhibition, the action of temperature in inhibiting respiration is extremely dependent on time of exposure (Gessner 1970). Mathieson & Burns (1971) and Fralick & Mathieson (1975) have shown that, due to physiological damage near the upper limits of temperature tolerance (26-32°C), anomalously high respiration rates are encountered in the red algae Chondrus, Gigartina and Polysiphonia. It is unlikely that the temperatures used in the present study were sufficiently high to produce this effect.

There was little evidence that respiration rate was correlated with taxonomic position. Thus, in Table 8.1, the rate for the green species Ulva was intermediate in the range of rates exhibited by the red algae. Likewise, in Britain (Table 8.3), Ulva again was intermediate although Enteromorpha had a very high rate. It has been suggested that the success of the red algae at the great depths (50-60 m) in the Mediterranean might be due to relatively low respiration rates (Larkum et al. 1967; Drew 1969) just as "shade" forms of higher plants have reduced respiration rates (Rabinowitch 1951, p 989). This hypothesis is not borne out by the present data with reference to Ulva, Sphaerococcus, Peyssonelia (organic weight basis) and Pseudolithophyllum (organic weight basis) at 53 m at Ganzirri (Table 8.1), where Ulva had the lowest rate on a dry weight basis and grew very well. A somewhat more meaningful measure of the significance of low respiration rates is seen when they are compared with, or expressed in relation to, the corresponding photosynthetic rates of the species. The ratio of photosynthesis to respiration (P:R) is commonly used (e.g. Ryther 1956a). The P:R ratio is useful in determining how close to compensation point a plant is. Since there is no certainty about the relationship of dark respiration to the rate of respiration in the light, it has been considered best to use net

photosynthesis:dark respiration for results from the oxygen method, and carbon fixation:respiratory carbon loss (converted from oxygen method results) for results from the ^{14}C method. This means that, assuming a daylength of 12 h, the value of P:R must be at least 1 (see Figure 9.15) if the photosynthesis of one day is to provide enough substrate, on balance, for one night of dark respiration (the ratio of gross photosynthesis:respiration will be 2). Short-term compensation points are less useful in an ecological context; if a plant is at or below compensation when in the light, then it will certainly be below when considering a full 24 h cycle. Values of P:R have been calculated from the in situ results at Ganzirri and are shown in Table 8.6A. Most notable among the values were, firstly, those for the calcified Peyssonelia and Pseudolithophyllym which were below 0.5 and the only species not having P:R in excess of 2 and, secondly, the values for Ulva which were consistently high, especially that of 9.3 at 53 m which was higher than the P:R for Sphaerococcus by a factor of four and those for Peyssonelia and Pseudolithophyllum by a factor of twenty. Thus this green species seems the best equipped of all studied for survival at great depth. Considering the equivalent values of the P:R ratio, using the ^{14}C results shown in Table 8.6B, there is a similar pattern except that the ratios are higher due to the higher photosynthetic rates indicated by the ^{14}C method. If it is assumed that the carbon fixation rate for Gracilaria is exceptional (there was no indication of such high rates in the oxygen method experiments) then Ulva again emerges with the highest values of P:R at the deep sites. Drew (1969) however, at Malta using the ^{14}C method in situ to measure photosynthesis and manometry to measure respiration, found that at 50 m, the red Peyssonelia had a P:R of 7.6 (c.f. 3.3 for this species in Table 8.6B) while the green Udotea had P:R 2.2 (c.f. Ulva, 26.5-35.5, Table 8.6B).

Table 8.6 Ratios of photosynthesis (P) to respiration (R) in Ganzirri algae.

A. Oxygen method, September. B. ^{14}C method, April and September.

A.

Species	Source depth m	$\mu lO_2\ mg^{-1}\ h^{-1}$ P	R	P : R
Laurencia	4.5	1.87	0.19	10.1
Gracilaria	4.5	0.93	0.15	6.2
Pterocladia	4.5	0.89	0.37	2.4
Vidalia	15	1.70	0.71	2.4
Sphaerococcus	4.5	0.33	0.13	2.5
	15	0.46	0.13	3.5
	30	0.48	0.13	3.7
	53	0.27	0.13	2.1
		$\mu gCcm^{-2}\ h^{-1}$		
Peyssonelia	53	0.60	1.75	0.3
Pseudolithophyllum	53	0.81	2.21[a]	0.4
Ulva	4.5	2.54	0.40	6.4
	15	2.22	0.15	15.2
	30	2.75	0.15	18.8
	53	1.36	0.15	9.3

B.

Peyssonelia (Apr)	60	3.18	0.97	3.3
Pseudolithophyllum (Apr)	60	4.72	2.21	2.1
Ulva (Apr)	4.5	10.90	0.31	35.5
(Sept)	4.5	3.63	0.40	9.2
(Sept)	53	3.87	0.15	26.5
		$\mu gCmg^{-1}\ h^{-1}$		
Sphaerococcus (Sept)	53	0.61	0.07	8.7
Gracilaria (Sept)	4.5	12.38	0.08	152.8

[a] April respiration value

In Britain considering the *in situ* ^{14}C experiments at Puffin Island (Table 8.7) very high P:R ratios were attained at 3 m, a maximum of 35.3 being shown for *Laurencia*, representing the red species, and an overall maximum of 48.5 again shown by the green *Ulva*. At the deep site, all six red species had values below 10 but *Ulva* had the very high value of 35.2.

Table 8.7 Ratios of photosynthesis (P, measured in situ, using ^{14}C method, Table 6.4) to respiration (R, measured in situ, Table 8.3) of British algae

Species	Source depth m	$\mu gC cm^{-2} h^{-1}$ P	R	P : R
Porphyra	0	6.34	.721	8.8
Rhodymenia	1	11.95	.495	24.1
Dilsea	4.5	6.57	.580	11.3
Enteromorpha	0	15.15	.927	16.3
Ulva	4.5	9.89	.204	48.5
Laurencia	0	5.72[a]	.162[a]	35.3
Dilsea	18	2.61	.9580	2.7
Delesseria	18	3.05	.627	4.9
Phycodrys	18	1.78	.287	6.2
Polyneura	18	0.59	.126	4.7
Nitophyllum	18	2.39	.370	6.5
Kallymenia	18	1.28	.174	7.4
Ulva	18	3.63	.103	35.2

[a] units - $\mu gC mg^{-1} h^{-1}$

In the surface experiments investigating the saturation characteristics of Rhodymenia, Delesseria and Polyneura (Figures 7.7 - 12) dark respiration measurements were made concurrently, and the P:R values are presented in Table 8.8A (using net photosynthetic rates at saturation).

Table 8.8 Ratios of photosynthesis (P) to respiration (R) from saturation experiments. A. Oxygen method, surface sunlight (see Table 7.3, Figures 7.7 - 12). B. ^{14}C method, tungsten-iodide (see Table 7.2, Table 8.4).

A.

Species	Source depth m	$\mu gCcm^{-2} h^{-1}$ P	R	P:R
Rhodymenia	1	6.30	0.31	20.3
Delesseria (red)	6	2.55	0.82	3.1
" (grn)	6	3.30	2.06	1.6
Polyneura	4.5	0.64	0.60	1.1
"	18	2.93	0.76	3.8
"	18	2.55	1.01	2.5

B.

Porphyra	0	18.0	0.91	19.8
Rhodymenia	1	32.0	0.82	39.0
Delesseria	9	11.9	1.26	9.5
Dilsea	9	22.5	2.40	9.4
Odonthalia	9	8.6	1.14	7.5

The upper sublittoral species Rhodymenia had a P:R close to 20, as in Table 8.7, but even though saturated, the sublittoral species Delesseria and Polyneura had low values of around 3. More specifically, the exposed and shallow specimens of these species had the lowest ratios around 1. Table 8.8B shows P:R ratios based upon the ^{14}C saturation rates attained in the laboratory saturation experiments (Figures 7.5-6). The ratios had high values due to the high photosynthesis noted in these experiments, but the pattern of high values in Rhodymenia and Porphyra and lower in the sublittoral species persisted. Heath (1969) states that at high photosynthetic rates, P:R may be equal to 10-20. In work on unicellular algae P:R ratios of 20 maximum have been recorded, decreasing to zero when nutrients were severely limiting (Ryther 1954, 1956a, b, c). In six littoral algal species representing all three major divisions, Kanwisher (1966) found the P:R ratio to be remarkably constant at 20 for saturation photosynthesis rates, and this value is of a similar order to those shown above for littoral and shallow species. It is notable that low P:R values predominated in the sublittoral algae (Tables 8.6, 7 and 8) whether incubated at saturation irradiance or in situ and this would appear to be a fundamental ecological and selective disadvantage. In the present work, only in one case (Rhodymenia, Table 8.8A) did P:R reach 20 when the oxygen method was used to measure photosynthesis. The very high values of P:R incurred using the ^{14}C method again underline the basic difference between these two methods.

It has already been stated that there is no certainty that dark respiration is a representative measure of respiration rate in the light. However, the level of irradiance at which the rate of photosynthesis balances that of respiration (i.e. the compensation irradiance) can be ascertained using the oxygen method, by noting at what irradiance the photosynthesis-irradiance curve cuts the x-axis (Figures/

(Figures 7.7 - 12). This irradiance is the "steady-state" compensation irradiance (Strickland 1958) and is of somewhat limited use in an ecological context, since it does not take into account the dark night hours. Thus, assuming a mean day:night of 12:12 hours, the steady-state compensation irradiance must be doubled (and, since photosynthesis increases linearly here, it will double too) to give the minimum irradiance necessary to promote survival. Table 8.9 presents the steady-state compensation irradiances for Rhodymenia, Delesseria and Polyneura interpolated from Figures 7.7 - 12.

Table 8.9 Compensation points of algae as measured by the oxygen method in attenuated surface sunlight

Species	Source depth m	Compensating irradiance $mWcm^{-2}$ PAR	Figure used
Rhodymenia	0	0.04	7.7
Delesseria (Red)	6	0.48	7.11
Delesseria (Green)	6	0.90	7.12
Polyneura	18	0.11	7.10
Polyneura	18	0.24	7.9
Polyneura	4.5	0.50	7.8

Due to its extremely high efficiency (p.225), Rhodymenia exhibited the lowest value, of 0.04 $mWcm^{-2}$ and the others were in the order of their photosynthetic efficiences (Table 7.5) i.e. Polyneura (18 m), Delesseria (red), Polyneura (4.5 m) and lastly Delesseria (green). Because of the high variability of respiration rates, the true value of compensation irradiance

is notoriously hard to compute, thus Kain et al.(1976) pointed out that the compensation irradiance of L.hyperborea was 0.75 $mWcm^{-2}$ (December) or 0.54 $mWcm^{-2}$ (July) as calculated by Jupp (1972), but only 0.13 $mWcm^{-2}$ (old frond) to 0.32 $mWcm^{-2}$ (new frond) as calculated by Lüning (1971). Using the ^{14}C method curves (Figures 7.4 - 6) and respiration rates from Table 8.4, compensation irradiances have been interpolated (as Jupp 1972) on the assumption that dark respiration is the same as respiration in the light, and these are shown in Table 8.10.

Table 8.10 Compensation points of algae as measured from gross photosynthesis measurements (^{14}C method) and dark respiration values (oxygen method)

Species	Depth source m	Compensating irradiance $mWcm^{-2}$ PAR	Figure used	Resp. rates used (from Table 8.4)
Rhodymenia	0	0.15	7.4	0.82
Porphyra	0	0.18	7.4	0.91
Delesseria	9	0.15	7.6	1.26
Dilsea	9	0.20	7.5	2.40
Odonthalia	9	0.72	7.4	1.14

Here the values for Rhodymenia and Delesseria were the same, being greater and less than, respectively, the corresponding values in Table 8.9. Considering both tables, only Odonthalia and the anomalous green Delesseria exceeded 0.5 $mWcm^{-2}$ and this would seem to be a reasonable upper limit for most species, implying a minimum mean irradiance, for daylight hours, of 1 $mWcm^{-2}$, to account for night-time respiration. Taking a specific

case, in Delesseria (red) at Dunstaffnage in August, the steady-state compensation irradiance was 0.48 $mWcm^{-2}$, daylength was 15 h, and assuming a sunny day, mean surface irradiance was approximately 15 $mWcm^{-2}$. Thus the fraction of surface irradiance required to produce 24 h compensation was,

$$0.48 \times \frac{24}{15} \times \frac{1}{15} \times 100 = 5.12\% ,$$

which, in coastal water type 3 (Figure 4.15A), as at Dunstaffnage, penetrates to 11 m. This is 5 m deeper than the depth at which the algae were collected. Carrying out similar calculations for Polyneura from 18 m at Puffin Island, with a daylength of 16 h and coastal type 1, 1.1% of surface irradiance would be required (from the compensation irradiance of 0.11 $mWcm^{-2}$ in Table 8.9) which (Figure 4.15A) penetrates to 30 m. Odonthalia collected in March at 9 m at Fife Ness had a requirement of 10% surface irradiance, available at 8 m in coastal type 3, one metre shallower than the source depth. Since all these calculations were based on relatively sunny conditions with mean surface irradiance of 15 $mWcm^{-2}$ PAR, they are necessarily maximum depth estimates, and can probably be halved safely to give minimum values, in which case, most of the algae studied appear to be living at depths to which they are only marginally adapted. Similarly, Kain et al.(1975) found that at the Isle of Man, only in June was the irradiance at 15 m above the lowest estimate of compensation irradiance (0.13 $mWcm^{-2}$) for L.hyperborea, whereas the species thrived at depths from 13-19 m. Further discussion on the interrelationships between depth, water clarity, time of year and physiology

will be presented in Chapter 9.

The results of the pretreatment experiment (Figure 8.14) showed that the measurement of dark respiration could be much modified by the prior history of the experimental material. The difference in SLA between the two pretreatments was presumably due to the increase in storage material in the light and decrease in the dark. Two possible explanations for the difference in respiration rates are, firstly, that the synthesis in the light-pretreated plants resulted in a disproportionate increase in storage or "metabolically inactive" material, thereby reducing respiration rate, and secondly, that the RQ (CO_2 evolved:O_2 taken up) was less in the dark-pretreated tissue, suggesting that some material less oxidised than carbohydrate was being metabolised. Supporting the latter explanation, it is known that the RQ of "starved" (i.e. tissue held in darkness and therefore unable to photosynthesise) leaves of terrestrial plants is higher than normal since at low levels of sugar, other substrates (i.e. fats and proteins) are readily drawn into the process of respiration (Yemm 1965). In this case, the low SLA of the more massive alga Dilsea might provide high reserves of respiratory substrate (carbohydrate), allowing respiration with a normal RQ to proceed for much longer than in Porphyra and Rhodymenia with their higher SLA values. The respiration of Dilsea showed the least difference of all three species, between light and dark pretreatments. Militating against this explanation, however, it should be noted that the rates for dark-pretreated material were closest to the rates normally attained (Table 8.4), whilst the light-pretreated rates were very low suggesting that it was they which had been most modified

by the pretreatment. The main purpose of the experiment was to investigate the effects of measuring dark respiration after indiscriminate pretreatments in in situ and laboratory experiments. Kanwisher (1966) drew attention to this also, but found on the contrary, that algae pretreated in the dark (either naturally by night, or artificially by day) had lower dark respiration rates than algae pretreated in the light and explained this as an "over-run" after effect of high "photorespiration" rates. The disparity between this result and the present findings may be explained by a further complicating factor, that of endogenous daily rhythmicity of respiration which was discovered to occur in Fucus sp. by Brown et al.(1955). The questions raised by this experiment confirm the views of Kanwisher (1966) and Šesták et al.(1971) on the desirability of using plants with a well-defined previous environmental history. This is clearly not an easy matter when using freshly collected field material.

CHAPTER 9

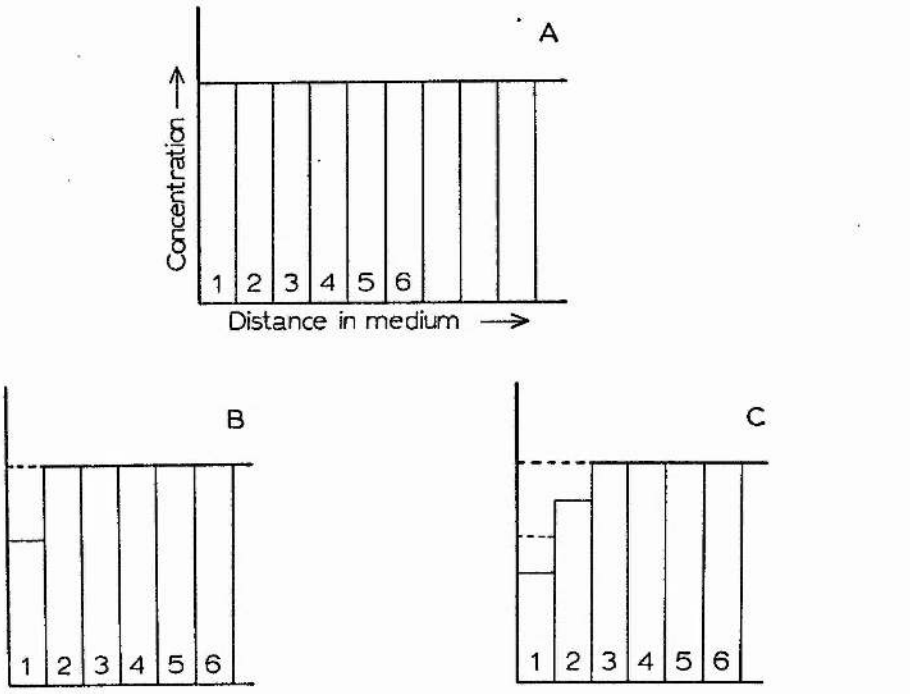
Some theoretical implications of the results

CONTENTS

1. Introduction

Although the majority of the experiments carried out in the present work were designed to investigate algal physiology, the field orientation of the project demands that the results be considered in relation to the gross growth patterns of the algae in the environment. Because of the wide variety of species studied, and of environmental conditions encountered, it is impossible to make this an exhaustive treatment; instead, some of the typical physiological results have been used to construct simple models which attempt to generalise the interactions between the physiology and ecology of the algae studied.

The first section deals with a problem which is probably more methodological than ecological, that of diffusive supply of external solutes under unstirred conditions. The experiments conducted in Chapter 3 posed the question of what levels of uptake by the plant during photosynthesis and respiration could be supplied by purely diffusive processes when there was no replacement of solute at the plant absorbing surface by other means, such as water movement. There are few treatments of this problem in the literature, especially with respect to marine algae where the medium, in nature, is regarded as being always exceptionally well-stirred (see e.g. Raven 1970). A computer method has been used here, its main advantage being that the progressive change in such parameters as solute concentration could be shown graphically for a large number of very short intervals of time.



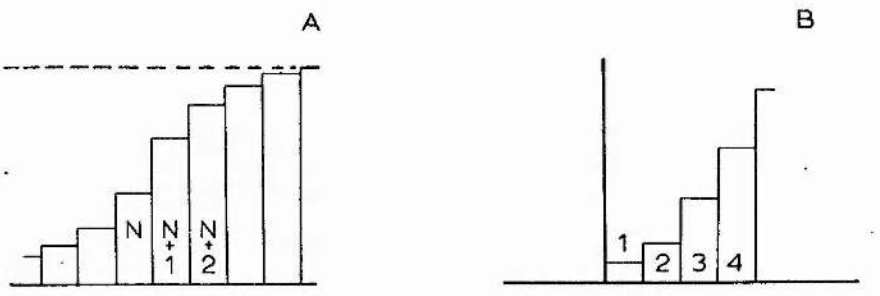


Figure 9.1. (upper). Schematic representation of concentration of solute in layers in the water column subtended by an algal absorbing surface; A, zero time; B, at end of timestep -1; C, at end of timestep -2.

Figure 9.2. (lower). As above; A, general case of three adjacent layers; B, special case of layer 1, adjacent to absorbing surface.

The second section deals with the interrelations between depth and photosynthesis. Much attention has been paid to this aspect of micro- and macroalgal physiology (Talling 1957; Kain 1966; Kain et al. 1976; Vollenweider 1965) and the results have been notoriously difficult to interpret. A computer model has been used here, again allowing the comparison of relatively large amounts of data.

In the third section, the relationship between measured rates of photosynthesis, and actual observed growth behaviour of algae has been discussed. Whereas growth analysis has been carried out comprehensively on several of the large Phaeophyta (Parke 1948; Neushel & Haxo 1963; Kain 1976a) there are few quantitative studies concerning Rhodophyta. In growth analysis, growth is defined as increase in dry weight of plants or stands of plants of plants during a time interval. Although this is basically what is done in studying photosynthesis, the results from the two types of study rarely correspond exactly for a variety of reasons. The purpose of the present theoretical treatment of growth has been to show whether the photosynthetic rates measured in the present study could result in the accumulation of dry weight observed in individual mature algae.

2. A computer model of diffusion-limited solute uptake

This simple model is based on the precepts of Fick's law of diffusion and attempts to investigate the theoretical limits of diffusive supply of oxygen and inorganic carbon to a plant surface in an unstirred medium. Figure 9.1A represents a plant absorbing surface (it is not relevant to the present discussion exactly what this surface is, in cellular terms) subtending a water column which has been divided into a series of layers of finite thickness, 1, 2, 3 etc., whose heights are proportional to the concentration of solution in them. At zero time, the concentration of the

solute concerned (in the present case, either O_2 or HCO_3^-) is the same in every layer. If it is now imagined that a finite interval of time has passed, "timestep -1", it can be seen (Figure 9.1B) that due to uptake by the plant surface, the concentration in layer 1 has decreased, whilst it is assumed that the other layers remain the same. Passing on to timestep -2 (Figure 9.1C) it is seen that more solute has been removed by the plant from layer 1, but also, layer 1 has been partially replenished, by diffusion, from layer 2, down the concentration gradient. If the timesteps and layer thicknesses were infinitely small, the model would replicate the diffusion system exactly. By using finite parameters, but of small dimensions, an approximation can be made using the computer to carry out the extensive claculations involved. The programme is shown in Appendix 2.

Fick's Law has the general form,

flux = diffusion coefficient (D) x gradient

so that, according to Weast & Selby (1967), if the concentration (mass of solid per unit volume of solution) at one surface of a layer of liquid is C_1 and at the other surface C_2, the thickness of the layer d and the area under consideration A, then the mass of the substance which diffuses through the cross-section A in time t is,

$$m = D \frac{A(C_1 - C_2)t}{d} \qquad 9.1$$

By assuming unit area, all subsequent calculations are simplified by the removal of the term A. If equation 9.1 is applied to the situation shown in Figure 9.1, the change in concentration of any layer can be calculated. Considering the general case of any three adjacent layers (see Figure 9.2A), N, N+1, N+2 which are part of a concentration gradient initiated by uptake by the plant, the change in concentration of layer N+1 can be calculated for one timestep as follows:

The influx of solute from N+2 is

$$m_{(N+2 \rightarrow N+1)} = \frac{(C_{N+2} - C_{N+1}) \times D \times t}{d} \qquad 9.2$$

The efflux of solute from N+1 is

$$m_{(N+1 \rightarrow N)} = \frac{(C_{N+1} - C_{N}) \times D \times t}{d} \qquad 9.3$$

total change in mass of solute in N+1 is

$$m_{(N+1)} = m_{(N+2 \rightarrow N+1)} - m_{(N+1 \rightarrow N)}$$

$$= \frac{(C_{N+2} - C_{N+1} - C_{N+1} + C_{N}) \times D \times t}{d} \qquad 9.4$$

original concentration of N+1 is

$$C_{N+1} = \frac{m_{N+1}}{\text{volume of N+1}}$$

and, because area = 1, volume = d, so

$$C_{N+1} = \frac{m_{N+1}}{d} \qquad 9.5$$

change in concentration of N+1 is

$$C'_{N+1} = \frac{m_{N+1}}{d} + C_{N+1}$$

which, substituting from equation 9.4 is

$$C'_{N+1} = \frac{(C_{N+2} - 2C_{N+1} + C_{N}) \times D \times t}{d^2} + C_{N+1} \qquad 9.6$$

Layer 1, situated next to the plant surface (see Figure 9.2B) is treated differently. If, at the chosen rate of uptake by the plant, the concentration in layer 1 becomes less than zero, the programme re-sets the concentration to zero and reduces the uptake by the plant to that which will maintain the concentration in layer 1 at zero. Thus,

$$C'_1 = C_1 - \frac{\text{uptake} \times t}{d} + \frac{(C_2 - C_1) \times D \times t}{d^2} \qquad 9.7$$

If $C'_1 < 0$ then uptake has been limited and, rearranging equation 9.7 using $C'_1 = 0$,

$$\text{uptake} = \frac{C_1 \times d}{t} + \frac{D(C_2 - C_1)}{d} \qquad 9.8$$

In order to assure a reasonable accuracy of the approximation it is necessary to use very small timesteps and layer thicknesses (i.e. volumes). Thus the total path length above the plant surface, taken as 1cm, was divided into 500 layers each of 0.002 cm (20 μm) thickness, and a timestep of 0.2s was used, allowing an incubation time of 40 min to be modelled during a run of 12000 timesteps.

The programme incorporated a plotting routine which plotted firstly, the concentration of solute in each layer at intervals of ten, one hundred and one thousand timesteps, and secondly the uptake at any instant was plotted as a continuous curve.

For an initial run, photosynthetic carbon uptake was modelled and an absolute maximum carbon uptake rate of 40 μg C $cm^{-2}h^{-1}$ was chosen, somewhat exceeding the maximum rate of 32 μg C $cm^{-2}h^{-1}$ recorded by the ^{14}C method in the present work (see Figure 7.4, Rhodymenia). A general value of the diffusion coefficient, $D=2 \times 10^{-5} cm^2 s^{-1}$ was used for all molecular species

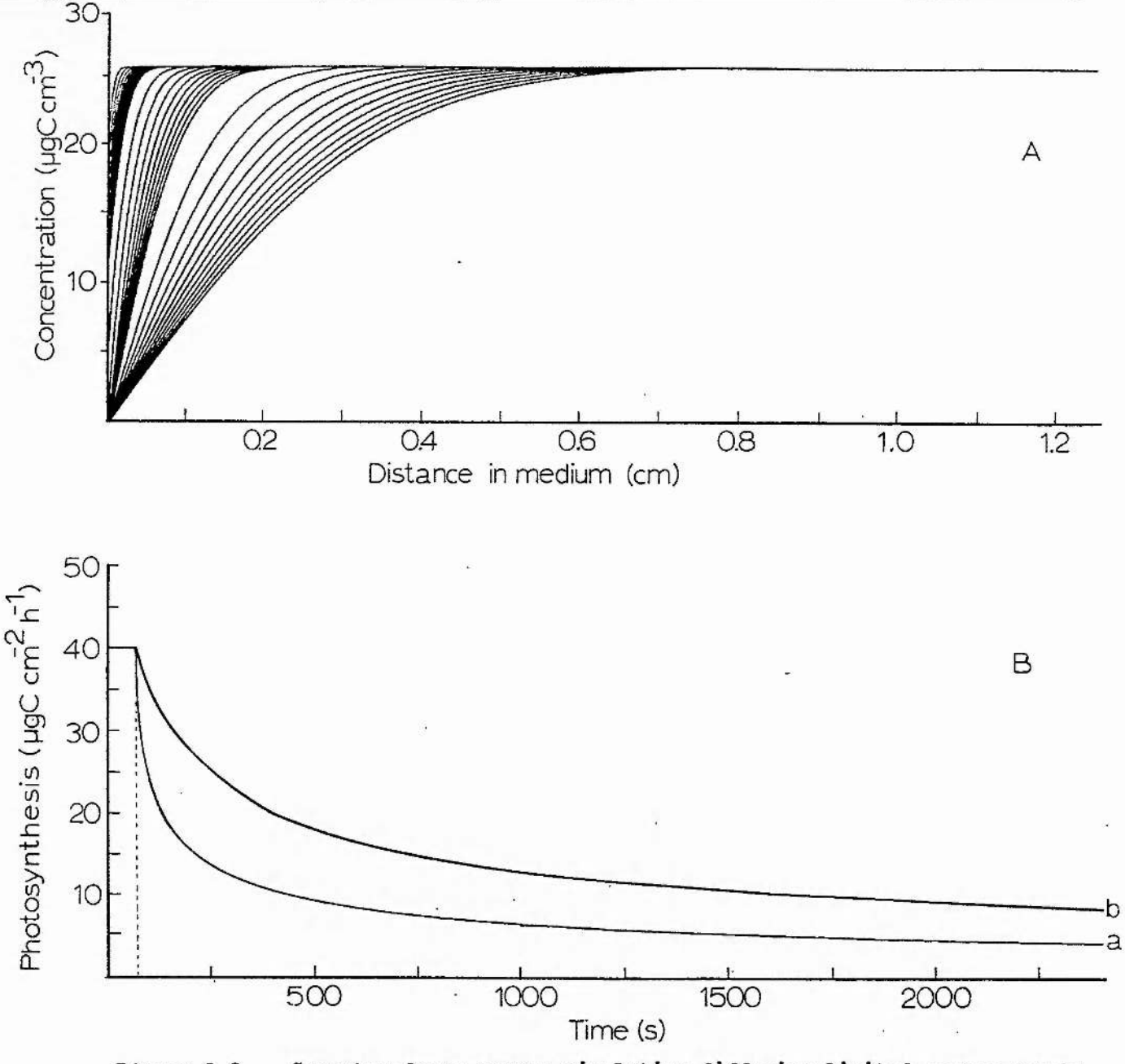


Figure 9.3. Computer-drawn curves simulating diffusion-limited carbon uptake by an algal absorbing surface with a maximum photosynthetic rate of 40 µgC cm^{-2} h^{-1}; A, inorganic carbon concentration profiles drawn at intervals of ten, one hundred and one thousand timesteps (1 timestep = 0.2 s); B, curve a, instantaneous photosynthetic carbon uptake rate, curve b, accumulated uptake rate.

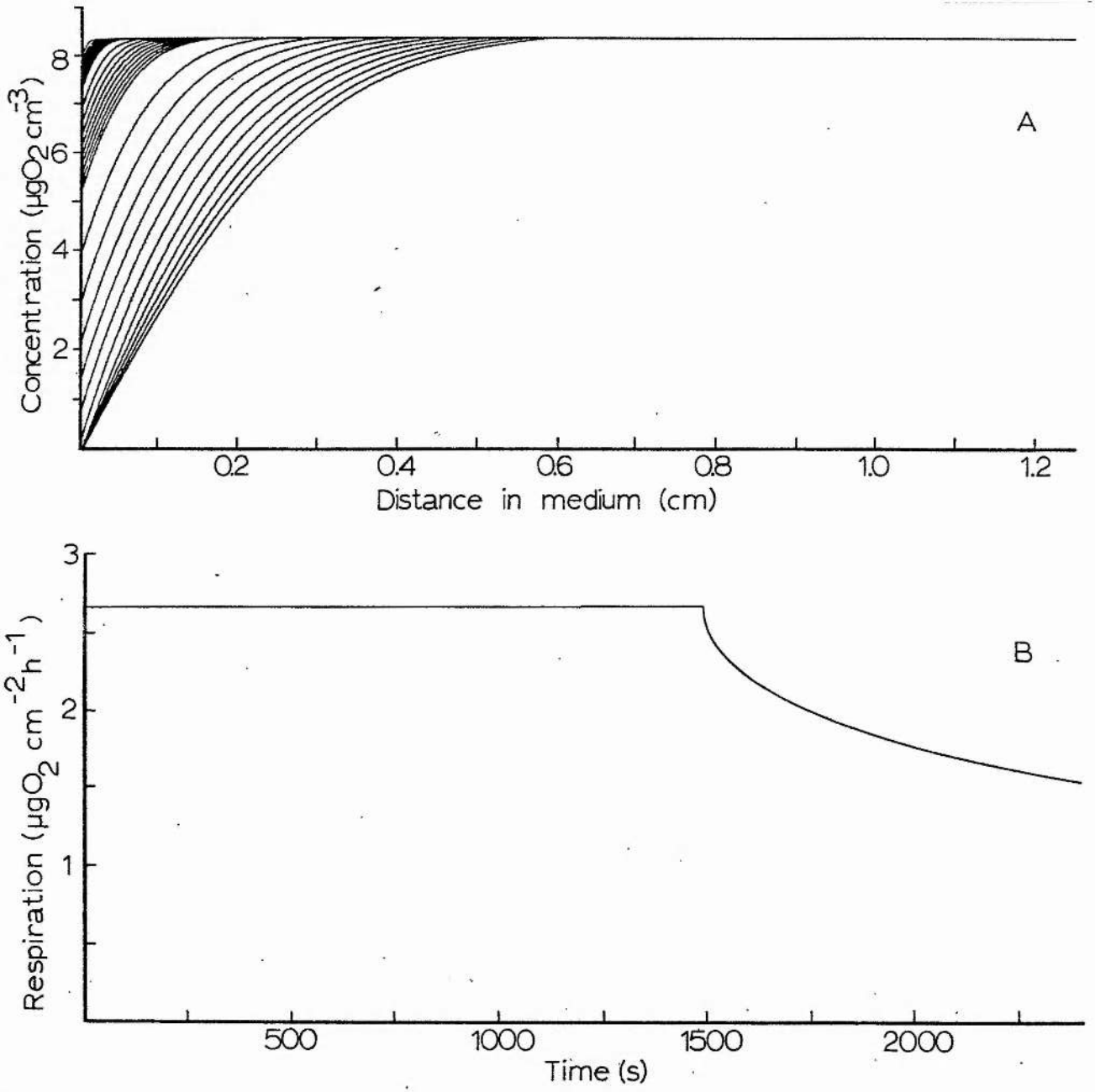


Figure 9.4. Computer-drawn curves simulating diffusion-limited oxygen uptake by an algal absorbing surface with maximum respiration rate of 2.66 µg O_2 cm^{-2} h^{-1} (≡1 gC cm^{-2} h^{-1}; A, oxygen concentration profiles at intervals of 10, 100 and 1000 timesteps (1 timestep = 0.25); B, instantaneous oxygen uptake rate.

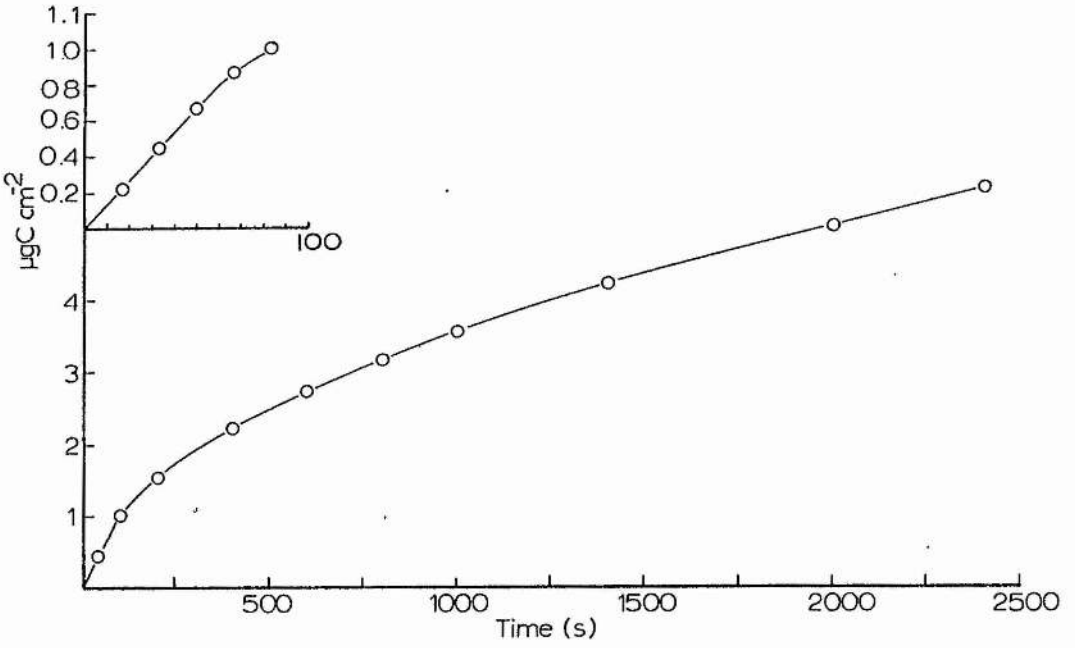
concerned in the programme runs described below (but see Chapter 3 p 88). Figure 9.3A, then, shows the concentration gradient in the water column above the plant surface up to 40 mins after the commencement of photosynthesis. The thickness of the "boundary layer", taken here as the distance from the plant surface to the point at which the concentration is 95% of the original, is seen to be 0.04 cm (400 μm) after 20s, 0.15cm after 200s (3.3 min) and 0.53cm after 2400s (40 min). Figure 9.3B (a) shows the possible uptake rate by the plant at any instant during the incubation, assuming an initial rate of 40 μg $cm^{-2}h^{-1}$. It is seen that after only 70s, the uptake rate begins to be limited by the low concentration of layer 1. At this point the boundary layer is 0.08cm (800 μm) in thickness. The uptake rate drops off rapidly to half of its initial value after only 150s, then undergoes a further slow decline to about 6 μgC $cm^{-2}h^{-1}$ at the end of the incubation period of 40 min. If the photosynthetic rate were measured by a cumulative uptake method, e.g. the ^{14}C method, the <u>mean</u> rate, as measured, would be in proportion to the area beneath curve a in Figure 9.3B (see also p.277).

A similar computer run was made simulating the measurement of dark respiration by the oxygen method. A rate of 2.66 μgO_2 $cm^{-2}h^{-1}$ (=1.86 μl O_2 $cm^{-2}h^{-1}$) was used as being close to the maximum recorded rates equivalent to 1 μgC $cm^{-2}h^{-1}$ (see Table 8.3), and the concentration of oxygen in the seawater was taken to be 8.3 μg$O_2$$ml^{-1}$ (=5.8 μl ml^{-1}). Figure 9.4A shows the concentration gradient produced during 40 minutes incubation and Figure 9.4B shows the possible uptake rate at any instant. It is seen that limitation of uptake again occurs, but due to the low initial uptake rate, not until 25 min have elapsed. At this point the boundary layer is 0.36cm in thickness.

The results shown by both of these trials, then, indicate that under perfectly static conditions, rates of the order of magnitude apparently attained by algae in the present study would result in the rapid development of boundary layers, and could not, in fact, be sustained for periods longer than about 40 min. The problem then becomes - what is the highest uptake rate which can be supplied by diffusive processes, for the time period of 40 min used at present in the model? To investigate this, the programme was modified so that for an arbitrarily chosen uptake value, the programme would run until limitation occurred. At this point the programme would stop and re-start using a new uptake rate which was half of the initial rate, and then, a) if the new rate was found to reach limitation within 40 mins, the programme again stopped and re-started using as a new uptake rate, half of the second rate, of b) if the new rate was found not to reach limitation, the programme stopped at the end of 40 min and re-started using as a new uptake rate, the mean of the first and second rates, e.g.

Initial rate 10 units	limitation
2nd rate = $\frac{10+0}{2}$ = 5 units	limitation
3rd rate = $\frac{5+0}{2}$ = 2.5 units	no limitation
4th rate = $\frac{5+2.5}{2}$ = 3.75 units	limitation
5th rate = $\frac{2.5+3.75}{2}$ = 3.125 units, etc.	

Thus, by trial and error, the programme "homed in" on the maximum sustainable initial rate, which, in modelling 40 min of photosynthesis with an inorganic carbon concentration in the medium of 26 μg Cml^{-1}, was found to lie between 6.53 and 6.86 μg $Ccm^{-2}h^{-1}$, or approximately 6.7 $\mu gCcm^{-2}h^{-1}$. This is quite a low value, considering also, that the incubation time of 40 min is less than used in nearly all experiments in the present work (but see below, p



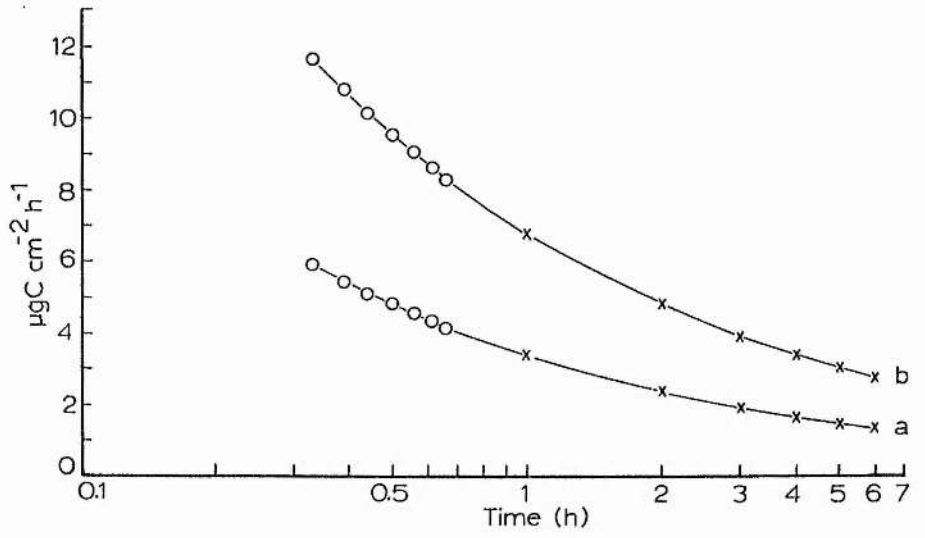
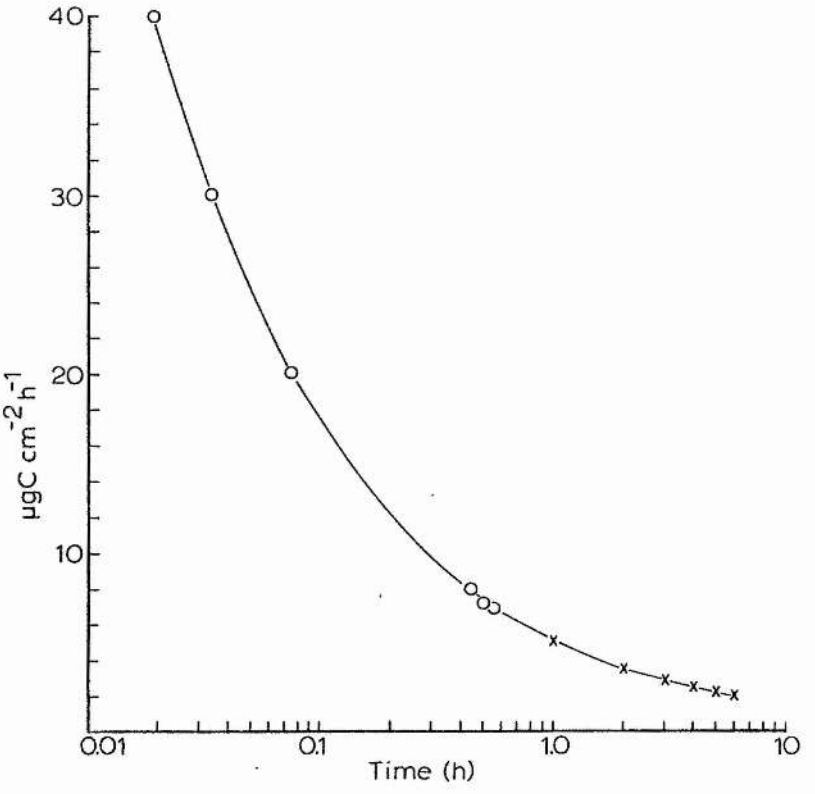


Figure 9.5. (upper). Accumulated uptake of carbon over a 2400s period (40 min), calculated for the case in Figure 9.3B (inset shows linearity up to 70 s).

Figure 9.6. (lower). Curves extrapolated (crosses) from the curves in Figure 9.3B (circles); a, instantaneous uptake; b, accumulated uptake (semilogarithmic plot).

As has been explained, the rates shown in Figure 9.3B curve a and Figure 9.4B are instantaneous uptake rates possible at relevant points in time during the incubation period, whereas uptake rates measured in the present work are based on cumulative uptake, measured at the end of the incubation period, divided by the length of incubation to yield a mean hourly rate. Therefore the programme was again modified to integrate the accumulated uptake at the end of each group of timesteps. These accumulated uptake values are shown in Figure 9.5, calculated for the example shown in Figure 9.3B. (The inset shows that accumulated uptake increases linearly as the rate remains constant, and begins to bend towards the abscissa when limitation occurs at 70s). These accumulated values, divided by the respective elapsed times, yielded a series of mean or cumulative rates as would be measured by either the ^{14}C or oxygen methods (see Figure 9.3B, curve b). It is seen that for most of the latter part of the incubation period, the uptake on a mean basis is about two times the possible uptake rate if measured instantaneously (Figure 9.3B, curve a). As time goes on, beyond 40 min, the mean rate will continuously approach the instantaneous rate, as the initial "burst" decreases in significance. Thus, although the mean rates measured on the basis of cumulative uptake are higher than the corresponding instantaneous rates, they are not really significantly closer to the initial rate.

Although the length of incubation time modelled was limited to 40 min due to restrictions on computing time, certain extrapolations can be made from curvilinear regression analysis of the data. Thus, a power curve ($y=a^x$) fitted to the data for the region above 1200 s of both curves in Figure 9.3B gave a r^2 value of 0.999998, which means that almost 100% of the variation of y is due to the curved regression on x (see Campbell 1967, p 298).



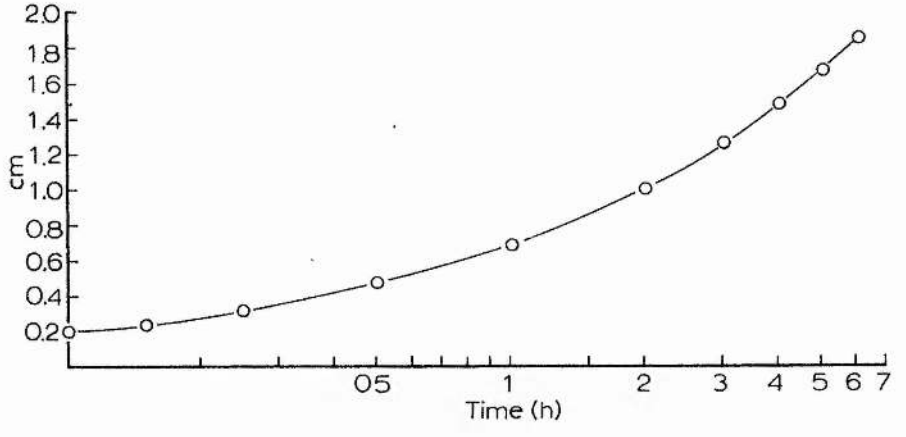


Figure 9.7. (upper). Maximum carbon uptake rates sustainable by diffusion alone for incubation times up to 6h. Curve extrapolated (crosses) from six computed limiting rates (circles) (semilogarithmic plot).

Figure 9.8. (lower). Progressive increase in thickness of boundary layer will time, for an initial carbon uptake rate of 40 µgC cm^{-2} h^{-1}. Curve extrapolated from data from Figure 9.3A (semilogarithmic plot).

The curves are shown re-drawn in Figure 9.6, extrapolated to 6h incubation time. The reduction of rate from the initial 40 $\mu gCcm^{-2}h^{-1}$ is seen to continue until, at 6h, the maximum possible instantaneous rate of uptake which could be sustained by a diffusive supply is only 1.36 $\mu gCcm^{-2}h^{-1}$ (curve a). If the rate had been measured by cumulative uptake (curve b) the value would be just over twice this, 2.63 $\mu gCcm^{-2}h^{-1}$. Clearly these rates give no indication of the magnitude of the initial rate.

A similar curve - fitting procedure was used to estimate the series of maximum carbon uptake rates which could be sustained by diffusive processes, without limitation, for incubation times up to 6h. Six arbitrary photosynthetic carbon uptake rates were taken, 40, 30, 20, 7.992, 7.218, 6.860 (the latter three from the "homing-in" programme) and the programme run in each case until limitation occurred, all within the 40 min incubation period. These six points were then plotted as a curve which forms the left-hand portion of the curve in Figure 9.7. A power curve fitted to these points had $r^2 = 0.999533$, again showing an extremely good fit. The curve was then extrapolated to 6h using the equation for this fitted curve. It can be seen from this curve that with an incubation time of 6h, the initial photosynthetic rate must be 2.02 $\mu gCcm^{-2}h^{-1}$ or less, if limitation is not to occur. (Since the rates on this curve are not limited, they are equivalent to uptake measured either instantaneously or cumulatively). Obviously, the extrapolation procedure is not entirely satisfactory, but taking values even at 2 or 3h, where the error is probably within 10%, rates of only around 3$\mu gCcm^{-2}h^{-1}$ could be expected if supplied by diffusion. The clear implication of these findings is that a rate measured at, say, 5h might be of the order of 2-3 $\mu gCcm^{-2}h^{-1}$ and this could represent either an initial uptake rate of 40 $\mu gCcm^{-2}h^{-1}$ (as in Figure 9.6) or a "true" continuous uptake of 3$\mu gCcm^{-2}h^{-1}$.

In the model, the maximum diffusion path was set at 1cm, but the reduction in concentration due to diffusion never in fact reached this limit during the 40 min of modelled incubation time, with the uptake rates employed (see Figures 9.3A and 4A). The extrapolations were made assuming that the diffusive supply continued at a constant rate, i.e. that the diffusion path was infinite. The extent of the seawater layer which is actually involved in the diffusive supply, the boundary layer, can be taken, as before, as the distance from the absorbing surface within which the concentration of the solute concerned is less than or equal to 95% of that in the bulk of the medium. The progressive increase in thickness of this layer with time can be found from Figure 9.3A for incubation times up to 40 min, for the extreme high rate of 40 $mgCcm^{-2}h^{-1}$, and several values for layer thickness so derived are shown plotted against time in Figure 9.8. Again, a power curve ($r^2 = 0.9987$) gave a good fit to the points and the curve was extrapolated, as shown, according to the equation. After 6h, the boundary layer has increased to 1.85 cm, and this would be the situation in the limited uptake case shown in Figure 9.6.

If the incubation conditions did impose a physical limitation on the length of the diffusion path (as might be the case with a tissue disc lying edge-wise in a 28ml bottle of internal diameter 2cm) then even greater limiting effects than indicated in Figure 9.6 could be expected due to the decrease in concentration of the unreplenished last layer in the series subtended by the plant tissue. However at this point it is necessary to realise the limitations of the model. The model attempts to simulate only diffusive supply to a unit area (say $1cm^2$) of plant surface from a direction normal to that surface. No account is taken of diffusion from the side, and, especially in tissue discs of relatively small diameter ($\sim$2.25 cm used in present work) this could be quite important since the

thickness of the boundary layer is in fact a function of the edge distance (Poole & Jenny 1971; p.91 this thesis). Also, it is not known to what extent unaccounted-for movement of water occurs in "static" bottles due either to very small physical movements of the bottles themselves (due to vibrations or external water movement) or to "micro" currents set up within the bottles due, say, to thermally or concentration-induced density gradients near the surface of the plant.

It is tempting to suggest that the shape shown by the uptake-time course curves in Chapter 3 (see Figure 3.3) was due to limitation of the type modelled here, however certain important differences should be noted. The curves in Figure 3.3 appear to reach a steady state after 2h whereas in Figure 9.6 for example there is still a marked decline in cumulative uptake rate (curve b) at this time. Also, in the "shaken" experiment (Figure 3.3D) in which no simple diffusive sitation would apply, an initial high rate was recorded as in the static cases. The long time-course in situ experiments conducted at Ganzirri did yield low rates (see Table 6.1) in general but the results indicated that photosynthesis was primarily irradiance-limited, which would not have been the case if serious limitation of inorganic carbon supply had occurred. Again, the re-plotted in situ results for Delesseria (Figure 7.20) show all the characteristics of an irradiance-limited photosynthesis curve obtained in shaken laboratory experiments. The unshaken experiments carried out in the field (see Figures 7.7-12) produced photosynthesis-irradiance curves which differed little from those obtained under shaken laboratory conditions. Figures 9.6 and 7 suggest that in a static experiment of 4 h duration, a rate greater than 4 μg $Ccm^{-2}h^{-1}$ is unlikely to be recorded. Referring to the Puffin Island in situ experiments (Table 6.4) it is seen that all rates recorded at 18m under presumably unshaken conditions, were in fact all around 4 $mgCcm^{-2}h^{-1}$, whilst rates obtained for algae

incubated at 3m, on the buoyant platform and therefore perhaps stirred to an extent, were considerably higher. However, Table 6.2 shows that in Ulva and Porphyra, rates much in excess of 4 μg $Ccm^{-2}h^{-1}$ were recorded in unshaken experiments conducted at Ganzirri in April. Also , Table 7.3 shows photosynthetic rates (from oxygen method) obtained under static conditions of around 8 $\mu gCcm^{-2}h^{-1}$ with a maximum rate of 17 $\mu gCcm^{-2}h^{-1}$ being attained by Rhodymenia. It seems most unlikely that these high rates could be attributable to rates which at the beginning of the incubation period were in excess of the 40 $\mu gCcm^{-2}h^{-1}$ used in the model, which is an absolute maximum value and greater than normally attained by most seaweeds (see Table 7.2) and also greater than rates recorded in shaken experiments.

However, there seems little doubt that some uptake rate limitation due to diffusion must occur in bottles incubated under static conditions, and the model shows how this may proceed. As suggested, edge effects and unaccounted-for water movements may reduce this limitation from that predicted by theory. Thus, although as has been stated, correction factors cannot strictly be applied to diffusion processes described by the model, it may be that they are applicable to the experimental situation. From experimental results obtained in Chapter 3 (e.g. Table 3.1, Figure 3.2) it would appear that measured photosynthesis rates (and respiration rates) are probably not lower than the "actual" by more than a factor of 2 or 3. In a similar situation, Drew (1972b) and Kain et al. (1975) used a factor of 1.5. Such factors are of a greatly different order than that of approximately 13 suggested by Figure 9.6 when initial rate is 40 μg $cm^{-2}h^{-1}$. It is thus suggested that rates measured in the present work may be regarded as being no less than one half to one third of rates measured using the same method but under agitated conditions.

Figure 9.7 generally summarises the findings of the model by showing the maximum possible initial uptake rates which can be sustained by a

diffusive supply (from one direction) for a series of incubation times. High uptake rates were quickly diffusion-limited and it was clear that there is no way of back-extrapolating from rates measured after a relatively long incubation time, to the initial rate in such a situation. This means that "correction factors" cannot be meaningfully applied to such rates. Curves of decrease in rate with time as produced by the model were similar to empirically-derived curves shown in Chapter 3, but were more extreme, implying the importance, in the experimental situation, of other factors such as edge effects and possible non-static conditions. In situ experiments gave results consistent with the hypothesis that irradiance was the prime limiting factor, and not diffusive supply.

Clearly the scope of the programme for modification is great. One particularly interesting treatment, however, would be to model diffusive supply to an absorbing surface situated in a flowing medium. This model would be a closer reflection of the natural situation in the sea.

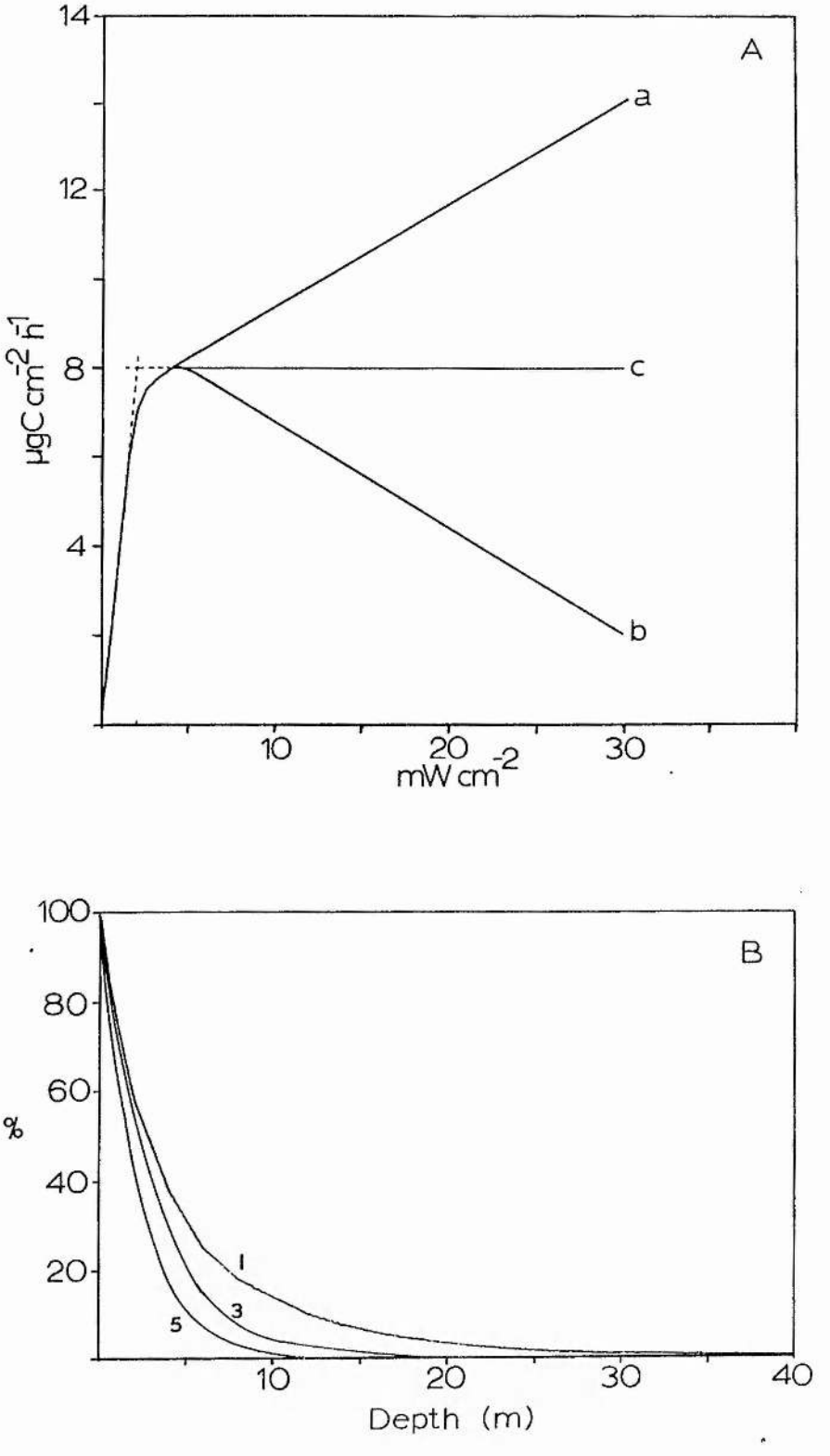


Figure 9.9. Curves used in computer model of the interaction between depth, time of year and photosynthesis; A, simplified photosynthesis - irradiance curves; a, sun alga; b, shade alga; c, intermediate alga; B, linear plots of attenuation of surface irradiance by waters of coastal types 1, 3 and 5.

3. A computer model of the interaction of depth and time of year with photosynthetic inhibition, saturation and compensation

Because of its effect on underwater irradiance, water depth is one of the prime factors influencing sublittoral algal zonation. Water clarity variations, and the changing daily march of solar irradiance during the year make the light ranges encountered in this environment among the most extreme of all plant habitats. As an extension of the photosynthesis - depth curves plotted in Chapter 6 (Figure 6.19) and the photosynthesis-irradiance curves in Chapter 7, an attempt was made to combine all three parameters by constructing a very simple model. Talling (1957) and Vollenweider (1965) have published quite complex mathematical models of the freshwater phytoplankton system based on photosynthesis-depth profiles, laboratory saturation curves, and irradiance-depth profiles. The present computer model seeks to represent three-dimensionally, photosynthetic rate of any plant for which a photosynthesis-irradiance curve is available, plotted against both depth and time of day. It also serves to produce, three and two-dimensionally, the saturation and compensation depths for the alga in question. A computer programme (Appendix 3) was devised which contained the following information in any one "run": (1) the photosynthesis-irradiance curve for one algal species, (2) the irradiance-depth curve for the water type concerned, (3) the irradiance-time-of-day curve for the water type concerned. Photosynthesis-irradiance curves (1) were obtained from the information in Chapter 7 and for this preliminary exercise, three simplified curves were used (Figure 9.9A) representing sun (curve a) shade (curve b) and intermediately adapted algae (curve c). It should be noted, however that all three curves had an I_k of 2mW (also that the theoretical I_k occurs before maximum photosynthetic rate is indicated, so that, in the computer extrapolations,

the depth at which saturation is said to occur, i.e. at which 2mWcm^{-2} prevails, is slightly shallower than the depth of maximum photosynthesis). The irradiance-depth curves (2) used were the light attenuation curves for Jerlov's coastal water types 1, 3 and 5 plotted on linear scales in Figure 9.9B. The irradiance-time curves (3) were diurnal solar irradiance curves which were entered into the program in the form of Vollenweider's (1965) equation (see equation 4.2) for the "standard light day" where noon maxima (term S_{tm}) were computed from the daily mean values of total irradiance of de Jong (1973) for different months in the north of Scotland. Values for daylength (N) were obtained for different months at latitude 58°N (i.e. Durness), from List (1951). The resulting curves for December, March and June are shown in the upper portion of Figure 9.13A.

Obviously the model relies on many very generalised assumptions, but in particular, the following cautions should be noted: (1) In its present form, the model probably approximates more closely to the experimental transfer experiments than to the natural situation because, for instance, shade plants (.e.g Delesseria) do not occur in an exposed position at 0m depth, (2) no account was taken of canopy shading, so the model portrays the irradiance environment of a canopy plant like L.hyperborea, or for smaller lithophytes growing in an area where L.hyperborea is absent due to other factors (e.g. substrate) (3) the abrupt change in slope at I_k in sun plants (as indicated in e.g. Porphyra, Figure 7.4) may be an exaggerated effect and the true situation would probably be a smoother curve (e.g. see Figure 7.3). (4) for simplicity, all three photosynthesis-irradiance curves had the same slope before saturation point. In reality this would probably not be so (see Figure 7.3) and this is a rather important point when considering compensation depth.

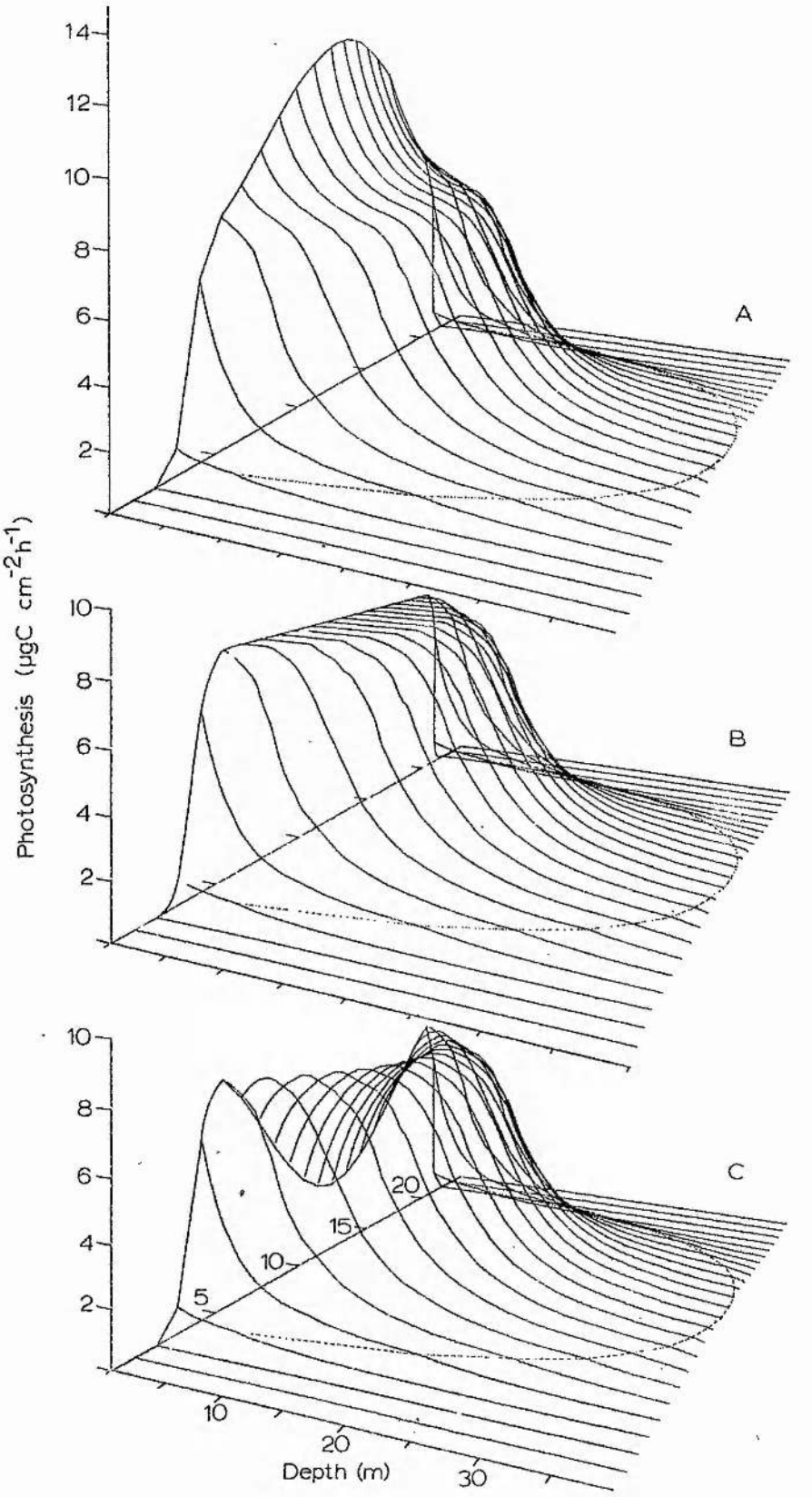


Figure 9.10. Three-dimensional plots drawn by computer of relationship between photosynthesis and depth and time of day for three algal types; the z-axis has the units - hours GMT; month June, site 58°N, coastal water type 1; A, sun alga; B, intermediate alga; C, shade alga.

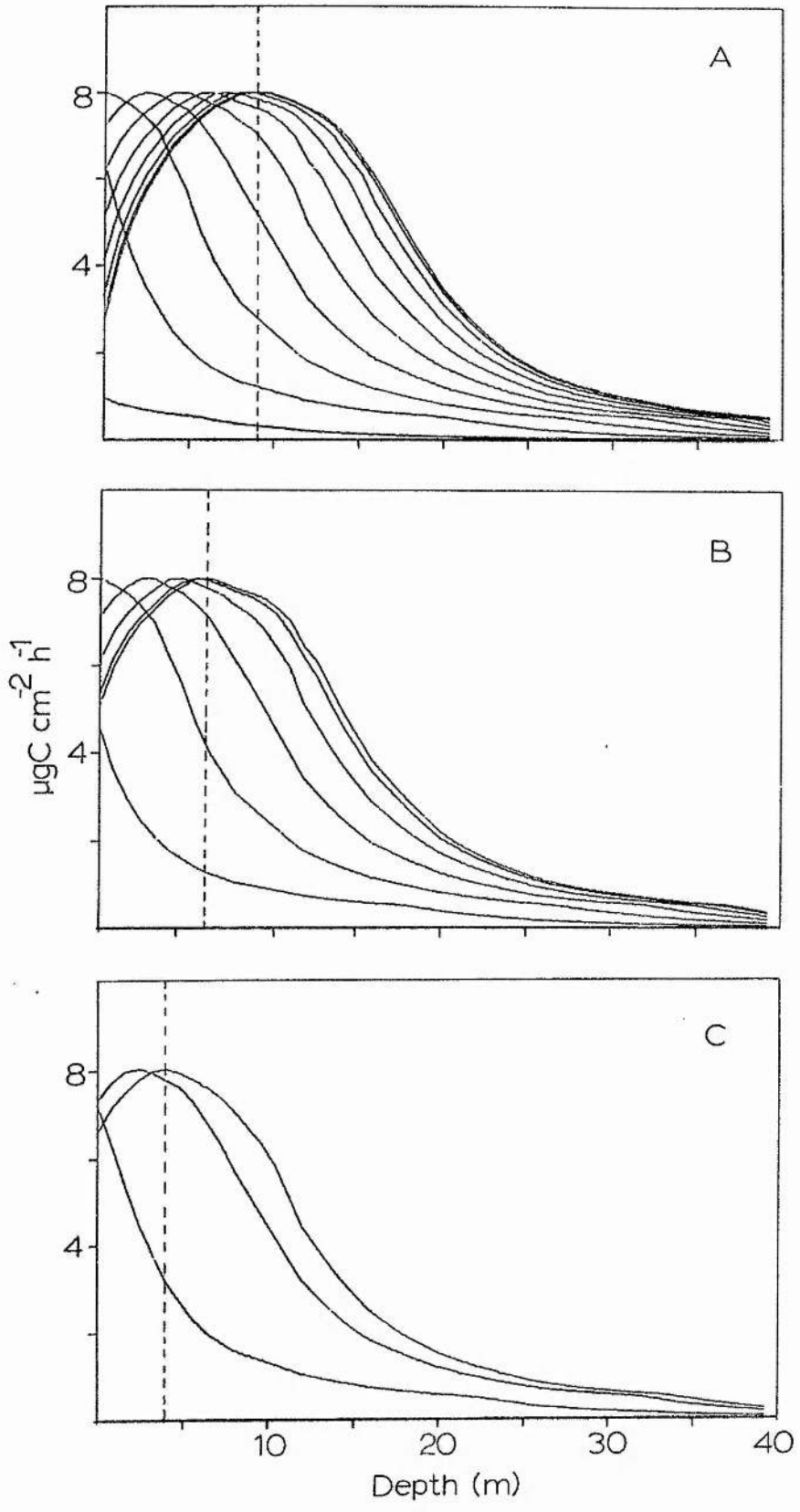


Figure 9.11. Two-dimensional plots drawn by computer of relationship between photosynthesis and depth and time of day for a shade alga, site 58°N and coastal water type 1; A, June; B, March; C, December.

The function of the programme was simply to plot photosynthetic rate with respect to depth and time of day, by combining the data provided concerning attenuation and changing surface irradiance. Figure 9.10 A,B,C, shows the three-dimensional relationship between photosynthesis, time of day and depth for the three algal types, one water type and one month. Figure 9.11A is the more usual two-dimensional family of curves showing this relationship for a shade alga, corresponding to Figure 9.10C. The photoinhibition of the shade plant near the surface is evident and it should be borne in mind that although this effect is regarded as a recurrent, non-harmful hazard in the respect of phytoplankton (Steemann-Nielsen 1974) it almost certainly implies lethality for photophobic red algae such as Delesseria. Thus such species would not be expected to survive unshaded at depths shallower than 9m (see Figure 9.11A). In March, the corresponding depth would be nearer to 6m (Figure 9.11B) and in December, 4m (Figure 9.11C), perhaps shallower, considering that water types are closer to coastal type 3 and 5 in winter. (These depths also depend upon the time-dependency of photoinhibitive effects, as suggested on p229 and in Figure 7.21, since single days of high irradiance can combine with coastal type 1 water clarity even in winter, to produce high irradiances in the sublittoral). The change in optimal depth with season poses the problem of the fate of sporelings of shade species derived from spores germinating in shallow water in the low irradiances of winter conditions and progressively exposed to higher irradiances as the season proceeds. However, several studies indicate that different ontological stages of any one algal species may have different optimal levels of irradiance. Thus Kain (1965) found that growth of early sporelings of L.hyperborea was saturated by an irradiance of 0.34 $mWcm^{-2}$ whereas photosynthesis of adults was found to saturate at 1-2 $mWcm^{-2}$ (Lüning 1971; Kain et al. 1975). Jones & Dent (1971) found that the growth of Dilsea

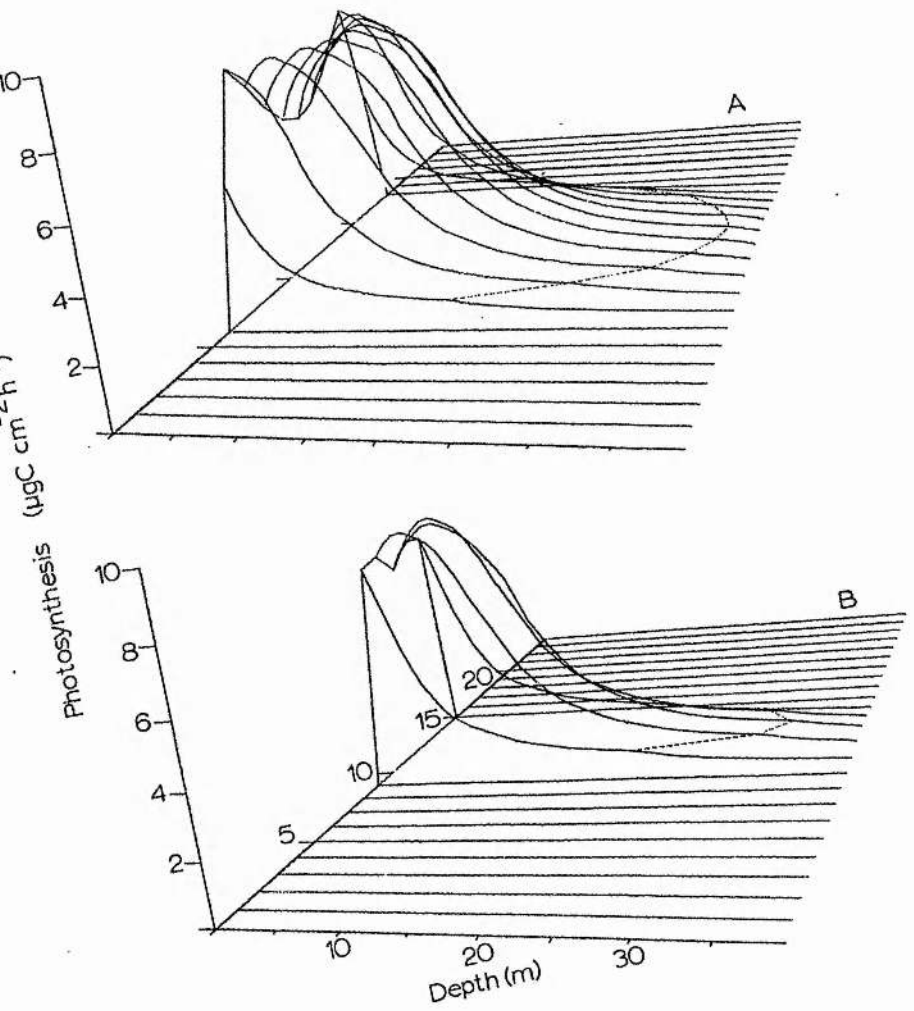


Figure 9.12. Three-dimensional plots drawn by computer of relationship between photosynthesis and depth and time of day for a shade alga, site 58°N, coastal water type 1; A, March; B, December. (cf. Figure 9.10C and Figure 9.11).

early sporelings was photoinhibited at irradiances as low as 0.3 $mWcm^{-2}$, with no growth occurring at 0.9 $mWcm^{-2}$, whereas in the present work no photoinhibition of photosynthesis was recorded in this species in irradiances of up to 10 $mWcm^{-2}$. Boney & Corner (1962) found that in young sporelings of Plumaria elegans, growth over a period of days under irradiances of greater than 0.1 $mWcm^{-2}$ was inhibited, but if adult algae were incubated for 24h at up to 1 $mWcm^{-2}$, no photoinhibition of photosynthesis was apparent. It thus appears that this intolerance to quite low irradiances may depress the upper depth limit of colonisation by sublittoral sporelings, producing a community in which the light tolerance increases with the seasonal increase in irradiance.

Considering the other curves, the "bump" in Figure 9.10A is due to the over-simplification of the sun plant curve as explained above and would be absent if a smooth curve had been used. If photosynthesis were directly proportional to irradiance level (i.e. if the photosynthesis-irradiance curve were a straight line through the origin) the photosynthesis-depth curve would follow that of irradiance as in Figure 9.9B. Figure 9.10B shows how an intermediate species would have a wide depth range over which maximum photosynthesis could be achieved. Figures 9.12A and B show how daylength at different seasons affects total daily photosynthesis by laterally compressing the three-dimensional curve.

The compensation point was taken, somewhat arbitrarily, but considering the results in Chapters 7 and 8, to be equivalent to an irradiance of 0.2 $mWcm^{-2}$ and gross photosynthetic rate (hence respiration rate) of 0.5 $\mu gCcm^{-2}h^{-1}$ (see Tables 8.9 and 10, and 8.3 respectively). The programme was designed to plot compensation depth as a dotted line on the three-dimensional curves at the depth to which 0.2 $mWcm^{-2}$ penetrated (see Figure 9.10). The daily march of compensation depth is also shown two-dimensionally in Figure 9.13A

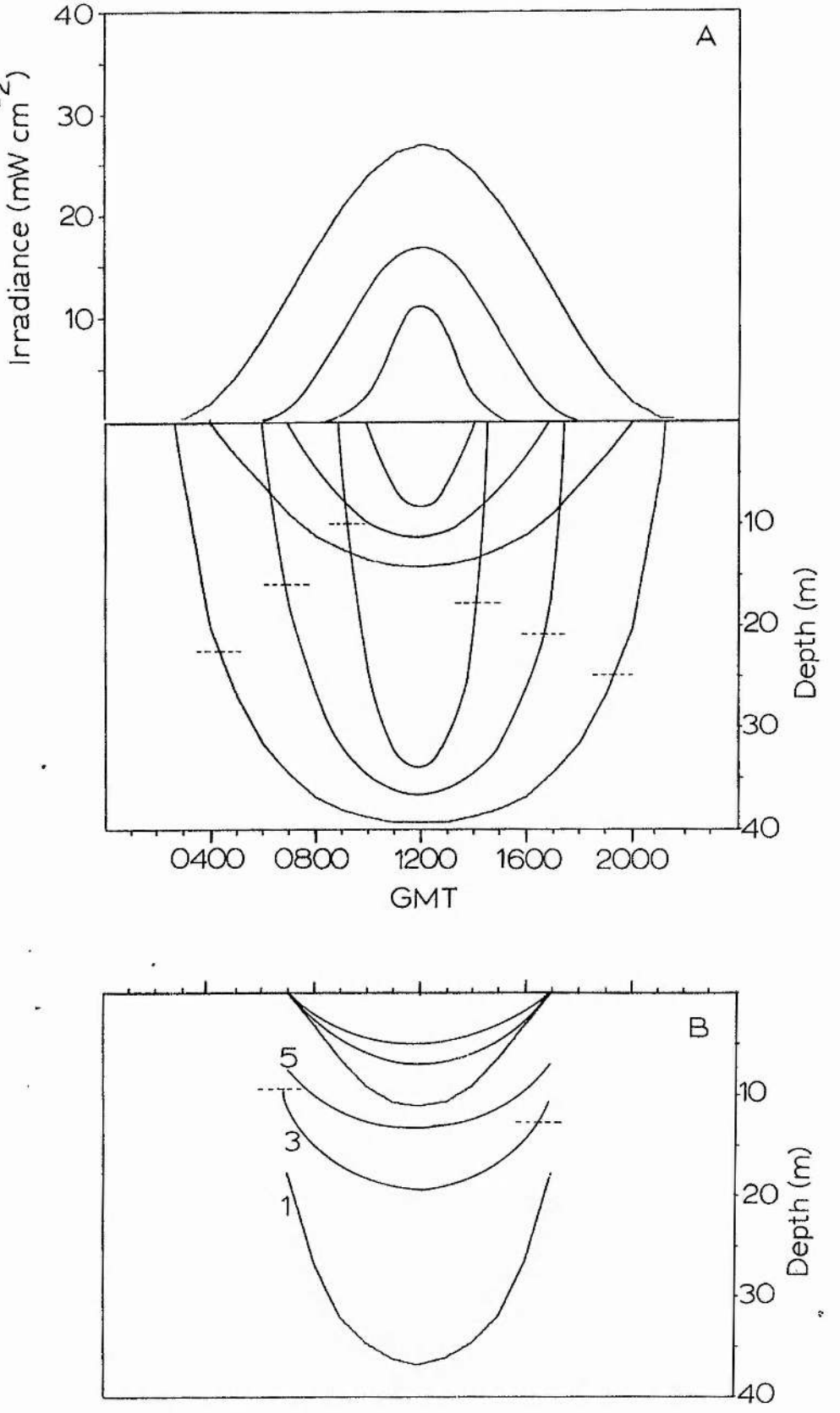
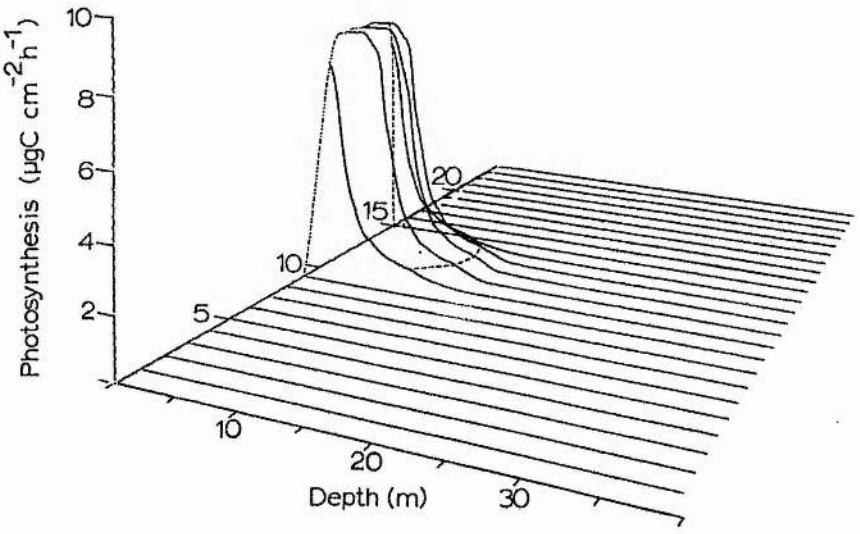


Figure 9.13A. (upper portion). Daily march of surface irradiance in June (daylength 19h), March (12h) and December (5.6h) for latitude 58°N; (lower portion). daily march of saturation (shallow curves) and compensation (deep curves) depth; dashed lines show, on right, the mean compensation depths for the photoperiod, and, on left, the mean 24h compensation depths; B, as above, for March, and coastal water types 1, 3 and 5.



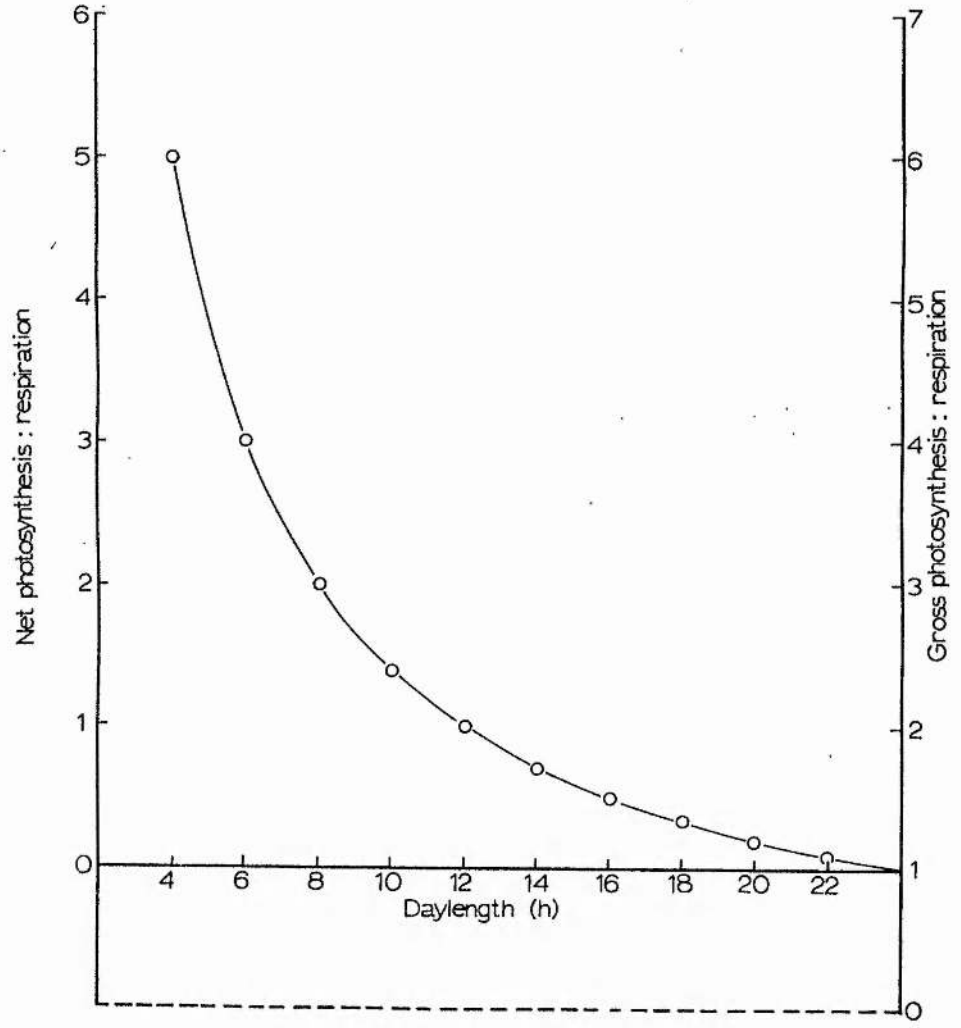


Figure 9.14. (upper). Three-dimensional plot drawn by computer of relationship between photosynthesis and depth and time of day for an intermediate algal type at 58°N, December, coastal water type 5.

Figure 9.15. (lower). Values of the ratios, net daily photosynthesis : respiration and gross daily photosynthesis : respiration required to produce the 24h compensation point, for a range of daylengths (i.e. photoperiods).

for December, March and June, together with the surface solar irradiance curves, and the corresponding curves showing the depths to which saturating irradiance ($2mWcm^{-2}$) penetrates. It can be seen that daylength has a much greater effect than noon maximum irradiance in modifying the amount of time an area of sea bed spends above compensation irradiance level. Even more important, with respect to maximum daily compensation depth, is the water clarity. Figure 9.13B shows compensation and saturation curves for potential (type 1), probable mean (type 3) and extreme (type 5) conditions in British coastal waters. The combined effect of short daylength and turbid water conditions on total daily photosynthesis is shown by a three-dimensional plot (Figure 9.14) for December and coastal water type 5.

The compensation depths discussed above were derived from a steady state or hourly basis and represent the daily march of compensation depth, at which negligible net photosynthesis takes place at the point of time under consideration. Alse marked on Figure 9.13A however, are the mean compensation depths for the photoperiod; these are the depths at which gross photosynthesis at the end of the daylight hours is equal to the respiratory loss over the daylight hours, i.e. the ratio gross photosynthesis : respiration = 1. In nature however, the plant must exist overnight also and the 24h compensation depth is the depth at which sufficient net photosynthesis occurs during the photoperiod to provide for respiratory loss at night (cf.p.264). The 24h compensation depth thus depends intimately on both daylength, and the ratio of mean gross photosynthesis : dark respiration. The curve in Figure 9.15 shows the value of this ratio (with the ratio of net photosynthesis : respiration also shown) which must obtain on average, during daylight hours at any depth at which a plant is to be above twenty-four hour compensation, for a range of daylengths. Thus, for a 12h photoperiod, the 24h compensation

depth is that depth where the ratio, gross photosynthesis : respiration =2, which is, in this case, twice the value required for compensation during daylight. In order, then, to achieve a ratio of 2, the photosynthetic rate must be doubled, which can be achieved by doubling the irradiance, since at these low levels, irradiance is directly limiting. The depth at which double the irradiance occurs is not of course, half the daylight compensation depth, since irradiance is attenuated exponentially; data must therefore be derived from a suitable attenuation curve for this water type (i.e. Figure 4.15A, type 1). The 24h compensation depths calculated in this way are shown for type 1 coastal water in December, March and June, in Figure 9.13A. Clearly, with long daylength, the 24h compensation depth is not much shallower than the mean compensation depth for the photoperiod in June. In December however, the 24h compensation depth decreases to 10m, almost half the mean photoperiod value. It is more likely that in winter and spring, waters would be of the clarity of coastal type 3 on average, and the 24h compensation depth in March, shown on Figure 9.13B, was found to be 9.5m. This is rather shallow in view of the communities of algae thriving at 18m at Puffin Island. Also, based on a compensation irradiance of 0.2 $mWcm^{-2}$, which is quite a low estimate, this depth is liable to be a maximum for the algal species studied here. As found in Chapter 8 (p.) and by other workers (Kain et al. 1976), the compensation depth indicated by theoretical means seems to be considerably shallower than the algal colonisation pattern would suggest. The 24h compensation depth of 23m in June was however consistent with the observed colonisation of algae at Puffin Island and it may be that, as suggested above, earlier growth stages have lower irradiance requirements. The situation is further complicated by the differential effect of temperature

on respiration and photosynthesis at limiting levels of irradiance, and this may offset the low irradiance during the winter by reducing the nightly respiration losses (see Strickland 1958; Kniep & Harder, cited by Lüning 1971).

Briefly, the model can be used to predict and show graphically in two and three dimensions, the response of a plant to environmental factors such as irradiance, depth and daylength, provided certain physiological data are available. In the present preliminary study, the three-dimensional figures showed well the extent of surface photoinhibition of photosynthesis in a susceptible "species" and the daily march of the phenomenon. It was also used to show the daily march of compensation depth, from which mean compensation depth for the photoperiod could be derived. With additional knowledge of respiration rates, 24h compensation depths could be calculated, but certain other factors, such as temperature, may be important here.

The model has great scope in that virtually any algal saturation curve can be used in conjunction with a variety of surface irradiance and attenuation data. Other potential uses include using the programme to model the production of transects of the whole underwater substrate rather than of parts of individual plants, as here. When integrated, the volume beneath such three-dimensional curves would then be proportional to daily yield of the sea floor per unit of length of the shoreline.

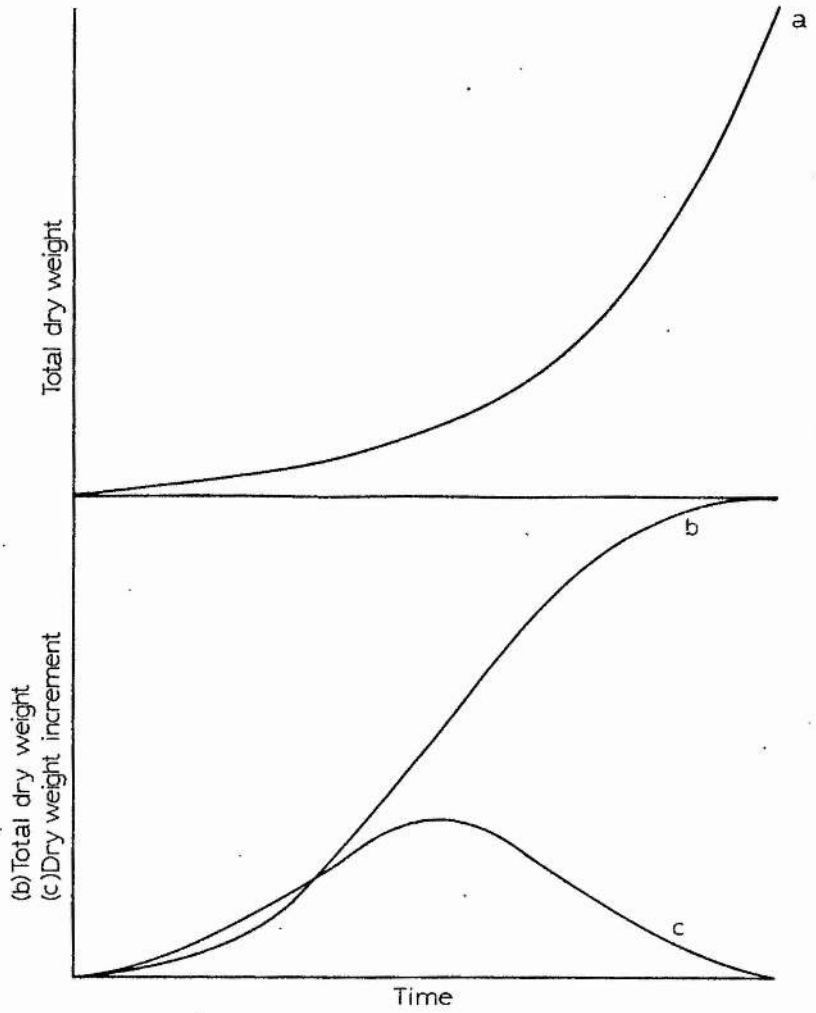


Figure 9.16. Plant growth curves; a, exponential growth; b, "sigmoid growth curve"; c, change in growth rate (total dry weight increment per unit time) concomitant with sigmoid growth.

4. The relationship between photosynthetic rate and relative growth rate

The growth of plants is frequently likened to the increase in capital of an invested sum of money, each part of which (each pound, say) accrues a certain proportion of itself per unit time (the interest). If a plant grows at a constant rate of cells produced per cell per unit time, or grams plant produced per gram plant per unit time, the actual increment added per unit time becomes increasingly larger in absolute terms and this results in an exponential increase in size. The phenomenon was named the compound interest law of growth by Lord Kelvin and was first applied specifically to plant growth by Blackman (1919) in the form,

$$W_1 = W_o\, e^{rt} \qquad 9.9$$

shown graphically in Figure 9.16 curve a, where,

W_1 = final weight (g) $\qquad$ r = rate of increase ($gg^{-1}d^{-1}$)

W_o = initial weight (g) $\qquad$ t = time interval (d)

e = base of natural logarithms ($\sim$2.72)

Because r, either in terms of cell number of dry weight, is expressed in relation to the amount of plant tissue present at any point in time, this type of growth rate is termed relative growth rate, or RGR. In a natural system, exponential growth obviously cannot continue unabated, and at some stage certain limiting factors come into play. In unicellular algal cultures, or in the well known seasonal phytoplankton rhythms, lack of nutrients becomes limiting resulting in a slowing of population growth rate (Pearsall & Loose 1937; Raymont 1963).

In individual specimens of macroalgae or higher plants, which can be regarded as

"colonial cell cultures" the genetical restraints of determinate growth are of importance in addition to environmental effects. These restraints impose a limit on growth which produces the familiar sigmoid growth curve, illustrated in Figure 9.16 curve b, due to changes in rate of growth, shown by curve c. The theoretical curves are necessarily mathematical approximations to the real-life situation and are likely to be much modified by environmental variations such as light and temperature occurring over the growth season. Such curves have nevertheless been approached by crop plants growing under relatively homogeneous environmental conditions (e.g. Thorne 1960). Complete annual growth curves for macroalgae have rarely been obtained due to the difficulties of culturing such plants to maturity, however, studies of the first 25-35 days of sporeling growth revealed that growth was exponential with a cell increase rate of 15% per day in Laminaria hyperborea (Kain 1965) and 5% per day in Plumaria elegans (Boney & Corner 1962). Such studies provide insight into growth patterns with respect to increase in volume and area but give no indication of dry weight increment, which can be wholly disconnected from the former. Boalch (1961) measured dry weight increment in excised shoots of Ectocarpus confervoides and found exponential growth at 11% per day (0.11 g g^{-1} d^{-1}) from 0-15 days, with a reduction in rate after that time. Studies of the seasonal growth of the annually renewed frond of L.hyperborea (on west coast of Scotland) by Robertson (1970) and Jupp (1972) revealed that growth on a dry weight basis was sigmoidal, almost ceasing in the summer months (June - October). Similar results were obtained by Mann (1972) studying L.longicruris, L.digitata and Agarum cribrosum. These species grew in length in an exponential fashion from October to April when a tail-off began with a decrease in rate setting in in May (experiments carried out in New Brunswick). In a study of the frond growth of a single specimen of

L.hyperborea (at Helgoland), Lüning (1971) found an exponential increase in area between February and June. Thus it appears that macroalgal growth follows a similar pattern to that of terrestrial annual plants, viz. an early exponential burst of growth slowing down at the end of the growing season, which appears to be around May - June in temperate waters.

Certain factors characteristically influence the size attained by a plant by the end of its growing season, and one such factor, important among land plants is the size of the propagule, if this represents W_o in equation 9.9. Thus, at the end of a trial of different maize varieties, Allison (1971) found that the largest plants were derived from the largest seeds. Theoretically, from equation 9.9, the ratio of final weights of two plants should be the same as the ratio of their initial or propagule weights, if both have the same growth rate. Few propagule weights for algal species have been reported in the literature, however, in a study of the settling rates of algal spores, Coon et al.(1972) measured the diameters and densities of red algal carpospores and from these, the respective fresh and dry weights can be computed (see Table 9.1). There was found to be a wide range of size, with the weight of the largest spore, that of Cryptopleura, being around thirty times the weight of the smallest, that of Callophyllis, and this ratio would obtain for these two species at any point in the growth period of plants propogated at the same time, if they had the same growth rates. Using the spore as a starting point and taking the measured photosynthetic rate as the growth rate, it is a useful exercise to calculate possible mature plant weight for comparison with weights actually attained by annual species. Firstly a measured photosynthetic rate must be converted to the term r in equation 9.9 in units of grams dry weight accreted per gram dry weight of plant per unit time, i.e. RGR. The conversion can be carried out in four steps involving several assumptions :

Table 9.1. Dimensions of red algal spores (from Coon et al. 1972).

Species	Diameter µm	Density or SG	Fresh[a] weight µg	Dry[a] weight µg
Cryptopleura	55	1.085	0.0945	0.0236
Myriogramme	45	1.079	0.0518	0.0137
Agardiella	38	1.181	0.0342	0.0086
Gelidium	26	1.136	0.0109	0.0027
Callophyllis	17	1.102	0.0030	0.0008

Specimen calculation, for Cryptopleura:

$$\text{spore volume} = \frac{4}{3}\pi r^3$$

$$= \frac{4}{3}\pi (27.5 \times 10^{-6})^3 \quad m^3$$

$$= 8.711 \times 10^{-14} \quad m^3$$

$$= 8.711 \times 10^{-11} \quad l \ (= 87 \text{ picolitres})$$

if SG=1, spore fresh weight $= 8.711 \times 10^{-8}$ g (= 87 nanograms)

if SG=1.085, spore fresh weight $= 9.450 \times 10^{-8}$ g

$= 0.0945$ µg

assuming 25% dry matter content,

spore dry weight = 0.0236 µg

[a] calculated data

1. Assume PQ and RQ both equal unity. (This has already been done, and rates derived from the oxygen method were taken from Table 8.8).

2. Using a mean photoperiod of 12h, subtract loss of carbon over 12h darkness from gain over the 12h photoperiod.

3. Use SLA to convert carbon fixation rate on an area basis, to dry weight basis.

4. Assuming ratio of total carbon : total organic matter content of plant = 0.45 (derived from a carbohydrate : protein ratio of 60:40 for Porphyra, Chapman 1970, p. 111) and organic matter content : total plant dry weight = 0.70 (see Chapter 5, p. 150) then proportion of total plant dry weight which is carbon is 0.45 x 0.70 = 0.32 (cf. value of 0.316 determined experimentally by Mann, 1972a, for Agarum cribrosum).

Taking Polyneura, studied at Puffin Island as an example,

hourly net photosynthetic rate = 2.55 $\mu g\ C\ cm^{-2} h^{-1}$

hourly dark respiration rate = 1.01 $\mu g\ C\ cm^{-2} h^{-1}$

daily net carbon fixation = (2.55 x 12) - (1.01 x 12)
= 18.48 $\mu g\ C\ cm^{-2} d^{-1}$

multiplying by SLA to express rate on a dry weight basis,

$$18.48 \times 0.450 = 8.316\ \mu g\ C\ mg^{-1}\ \text{dry wt.}\ d^{-1}$$

expressing carbon fixation as dry weight accretion,

$$\frac{8.316}{0.315} = 26.4\ \mu g\ \text{dry wt.}\ mg^{-1}\ \text{dry wt.}$$

which represents a RGR of 0.0264g $g^{-1} d^{-1}$

Since this rate was derived from a saturation photosynthetic rate, it represents a potential RGR which is probably greater than the actual value. If, then, we assume that a spore of Polyneura germinates in winter and the adult plant is harvested after six months, the growth season (t) will be approximately 180 days. The spore dry weight (W_o = 0.0236 µg) used in the

calculation below is that of Cryptopleura (see Table 9.1) an alga closely related to Polyneura. Thus,

$$W_1 = W_o \, e^{rt}$$

$$= 0.0236 \times 10^{-3} \times 2.718^{0.0264 \times 180}$$

$$= 2.74 \text{ µg dry wt.}$$

This is an extremely low value and represents a plant having an area of only about $0.0012 cm^2$. Polyneura plants collected in July at Puffin Island were frequently over $500 cm^2$ in area and 1g dry weight. Also, measurements of plants in July are liable to be minimal assessments of the plants' production over the growing season. Work by Mann (1972) on Laminaria longicruris indicated that the biomass of algae at the end of the growing season might be only one fifth of the total seasonal production, due to physical loss of tissue at the distal end of the thallus. However, if, for the present, the dry weight of an adult plant of Polyneura is taken as 1g, the RGR required to produce this from a spore of dry weight 0.0236 µg in a period of 180 days can be calculated using a modification of equation 9.9, thus,

$$W_1 = W_o \, e^{rt} \qquad 9.9$$

$$\log W_1 = \log W_o + rt \log e$$

and, since $\log_e = 1$,

$$rt = \log_e W_1 - \log_e W_o$$

$$r = \frac{\log_e W_1 - \log_e W_o}{t} \qquad 9.10$$

Substituting the above values for W_1, W_o, and t,

$$r = \frac{\log_e 1 - \log_e (0.0236 \times 10^{-6})}{180}$$

$$= 0.0975 \text{ g g}^{-1}\text{d}^{-1}$$

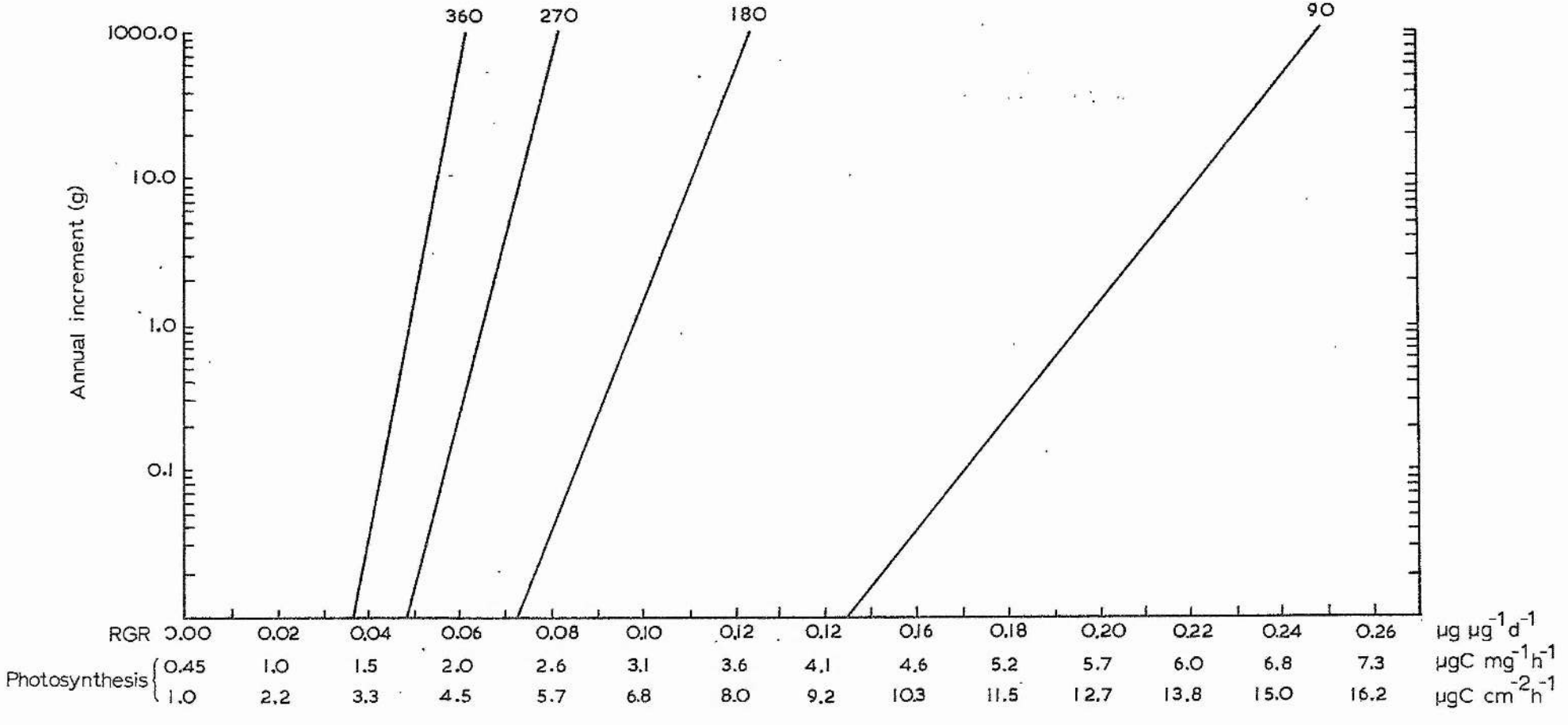


Figure 9.17. Relation of annual yield to RGR for growth seasons of 90, 180, 270 and 360d (semilogarithmic plot).

This is about 3.5 times the RGR as derived above from the photosynthesis measurements, and itself represents an hourly net photosynthesis rate of approximately 6.7 μg C $cm^{-2}h^{-1}$ (assuming the same respiration rate). Using the oxygen method, rates as high as this were attained only by Rhodymenia, with an hourly net rate of 6.3 µg C $cm^{-2}h^{-1}$ (see Table 8.8) representing a RGR of 0.0571g g^{-1} d^{-1} (assuming SLA of 0.25 cm^2mg^{-1}). Clearly, if as Mann (1972) found, the final weight of 1g really represents a production of up to five times this amount, even higher rates of photosynthesis and higher relative growth rates would be necessary during the growth season. Measurements with the ^{14}C method consistently produced higher rates of photosynthesis than the oxygen method. Thus, a maximum rate under the "ideal" laboratory conditions of high irradiance and shaken incubation bottles was recorded again for Rhodymenia, to be 32 µg C $cm^{-2}h^{-1}$ representing a RGR of approximately 0.3g $g^{-1}d^{-1}$ which would be more than enough to produce 1g in 180 days. In order, then, to compare the relation of annual yield to RGR, a series of curves was plotted semilogarithmically, for four different values of t, in Figure 9.17. It is seen that due to the exponential nature of the relationship, for the higher growth rates and especially with a long growth season, yields are greatly increased for relatively small increases in the value of r. Thus, although a RGR of 0.0975 is required to produce a mature plant of 1g dry weight, in 180d, an increase to an RGR of only 0.1085 would produce a plant of 7g, which was the weight of the exceptionally large individual of Polyneura reported in Chapter 5 (p. 140). Although most algae incubated in the laboratory and at shallow sites had rates of photosynthesis which could produce reasonably sized plants within a 180 day growth season, most deep algae when incubated in situ (see e.g. Table 6.4.), and the Ganzirri plants (Table 6.1.) did not.

This implies that either (1) measured rates were artificially low, or (2) the measured photosynthetic rates were low due to the advanced stage of the season, and occupied the latter part of the sigmoid growth curve. It has already been suggested that in situ rates might be rather low due to lack of agitation of the incubation bottles, however, the fact that higher rates were noted in spring at least at Ganzirri, provides some evidence for the second explanation, and implies that high photosynthetic rates over a comparatively short part of the growth season may contribute to the major portion of the final biomass of certain species. Of course, certain other factors are critical in the production of net growth, such as the ratio of photosynthesis to dark respiration, which governs the proportion of material lost each night. However, it should be recalled that, since Figure 9.17 was based on the maximum spore size recorded by Coon et al. (1972), the rates on the abscissa required to produce the final weight shown are probably minimal and thus any sporeling starting out from a spore of smaller size would require to have a correspondingly higher growth rate.

In the above example, the species under consideration, Polyneura, was an annual, and the spore weight, W_o, was a very small quantity in biomass terms. Certain prominent members of the sublittoral flora are perennial however, notably L.hyperborea but also such red species as Delesseria and Ptilota. Delesseria may persist for 5-6 years (Dickinson 1963), the perennating parts being the frond midribs which, divested of the membranaceous part of the thallus, produce new fronds in the early spring. The dry weights of these organs, may be of the order of a few grams and it is clear that the initial mass (W_o) to be considered here in computations of equation 9.9 is several orders of magnitude greater than the mass of a spore. Even a passive diffusive supply of soluble carbohydrates to a frond "primordium" would constitute a great source of

energy to an organ which at this initial growth stage would have a low light receiving area. In this connection, Kolkwitz (1900, cited by Fritsch 1945, p. 534) found that in winter, Delesseria midribs had a high starch content which decreased progressively with the development of new fronds in spring. Translocation of metabolites is known to occur in the Phaeophyta (in Macrocystis and L.hyperborea, see Clendenning 1971 and Kain et al. 1976) and to aid new season's growth, and may thus also be important in perennial Rhodophyta even although true conducting tissues are absent. Further critical experiments are required to clarify this point. There are indications (Jupp 1972; Kain et al. 1976) that annual brown algae like Saccorhiza polyschides may have significantly higher photosynthetic rates than true perennials like L.hyperborea and this is consistent with the present suggestion that spore-propogated specimens may require intrinsically high growth rates. This did not however appear to be a consistent trend in the present work (see p.190). Among the red seaweeds, indeed, it is not at all clear which species are true perennials and which are not. Knight & Parke (1931), in addition to annuals and perennials, designated as "pseudoperennials" (Dixon, 1973, preferred "pseudoannuals") those algae in which the thallus was "almost completely" removed at the end of its growing season (usually autumn) and re-growth was due both to sporelings and to proliferation of microscopic basal fragments of old plants. In the present study, although in species such as Delesseria there was always clear evidence of a plant being older than one season, in species such as Polyneura, with no well-defined perennating parts, either annual or pseudoperennial specimens may have been used. It seems unlikely however that within a species, photosynthetic rates of one-year-old specimens would be different from rates attained by older plants.

In summary, then, three intrinsic factors were found to influence the plant's annual yield : (1) Initial mass (W_o) of propagule,

(2) relative growth rate, RGR (r), (3) length of growing season (t). Due to the exponential nature of growth, at least at certain ontogenetic stages, quite small changes in any one of these factors could dramatically influence the annual yield. The *in situ* measured rates of photosynthesis reported in this thesis were frequently too low to account for the observed annual yield of the sublittoral algae studied, if these algae are true annuals and are spore-propogated. Species propogated from larger, perennating, organs such as overwintering midribs (*Delesseria*) could easily attain the recorded yields, growing constantly at the measured growth rates.

CHAPTER 10

General Discussion

In absolute terms, the photosynthetic rates attained by some of the algae in the present work were as high as the maxima recorded in the literature. When the ^{14}C method was used, under agitated conditions, Rhodymenia attained a rate of around 30 µg C cm^{-2} h^{-1} and Dilsea of 20 µg C cm^{-2} h^{-1}. These are only slightly lower than rates recorded for the generally highly productive brown algae; 40 µg C cm^{-2} h^{-1} for Macrocystis (Clendenning & Sargent 1957) and 35 µg C cm^{-2} h^{-1} for Saccorhiza polyschides (Kain et al. 1975). In the field, under static conditions, with the ^{14}C method, rates of 20 µg C cm^{-2} h^{-1} were attained by Porphyra in June at Durness, and 11 µg C cm^{-2} h^{-1} for Rhodymenia in July at Puffin Island. If these rates are doubled to allow for stagnation effects (Chapters 3 and 9) due to lack of agitation, they would represent rates of 22-40 µg C cm^{-2} h^{-1}, similar to those in the laboratory.

Although these rates are high as far as seaweeds in general are concerned (see also Tables 6.11 and 7.2,3) they are in fact within the range of 11-44 µg C cm^{-2} h^{-1} given by Sestak et al. (1971, p.31) for net photosynthesis in "terrestrial herbs from shaded habitats". In cultivated plants ("sun" plants) with the Calvin cycle, rates can approach 100 µg C cm^{-2} h^{-1} and in plants with the C_4-dicarboxylic acid cycle, rates up to 200 µg C cm^{-2} h^{-1} can be attained. In the present work, rates attained using the oxygen method were significantly lower, the highest recorded being equivalent to 6 µg C cm^{-2} h^{-1} net photosynthesis (using PQ = 1) in Rhodymenia under static conditions, implying a possible net rate of 12 µg C cm^{-2} h^{-1} if shaken. However this is still quite a high rate of net photosynthesis, compared with the findings of other authors (Table 7.3).

Considering the rates attained in the Mediterranean, at Ganzirri the maximum rate recorded in unshaken conditions using the ^{14}C method was very high, 18 µg C cm^{-2} h^{-1} but for the oxygen method the highest rate attained, 2.75 µg C cm^{-2} h^{-1} by Ulva, was lower than the lowest net rate quoted by Sestak et al.as typical for terrestrial plants, 8 µg C cm^{-2} h^{-1}, for shade leaves of evergreen broadleaved trees.

Since photosynthesis is the producing process carried out by plants it is clear that its control by the availability of external factors must have an important involvement in the competition between the algae, and their consequent distribution (see Bidwell 1974, p. 592). The importance of irradiance in controlling photosynthesis was established by the underwater experiments. Thus, transfer of shallow-growing algae to deeper habitats resulted in reduced photosynthesis due to the attenuation of irradiance by the water column, and conversely, deep algae showed inhibition due to excess irradiation, when transferred to the shallows. At Ganzirri, a study of Sphaerococcus and Ulva in situ at four depths showed that, even without transfer, lower rates were attained by algae living at extremes of the depth range.

The limitation of the photosynthesis and perhaps colonisation of algae at the lower limit of the photic zone can thus be explained in terms of limitation of "supply of energy" in the sense used by Blackman (1905) in his paper on "Optima and limiting factors", and earlier, by von Liebig in his "law of the minimum" (see Odum 1959, p.88). In addition to being limited by minimal supply of a factor, organisms can be limited if it is present in excess. Odum (1959, p.88), in considering the ecological action of limiting factors, regarded organisms as being limited at one end of their range by scarcity of a factor, by Liebig's "law" of the minimum, and at the other end by their tolerance to an excess of this factor, citing Shelford's "law" of tolerance. Limitation by excess of a factor probably is exemplified by

the surface photoinhibition of deep-living shade algae such as Phycodrys, Delesseria and Sphaerococcus. Thus, the truly sublittoral algal species (although they do survive also in fortuitous shade niches in the littoral zone) are limited in their upward colonisation by their tolerance of high irradiance (perhaps linked with spectral effects, such as UV radiation) and in their downward colonisation by their efficiency of use of limited quantity (again spectral quality may also be of importance) of this same commodity - this is sometimes known as "shade tolerance" (e.g. Spence & Chrystal 1970a & b). The reduction of irradiance caused by the dense canopies of stratified communities e.g. Laminaria hyperborea "forests", may allow extension of the normal upward limit of colonisation by shade species existing in the "underflora".

At extremes of the irradiance range, physiological adaptations to high and low irradiance were found to occur in such species as Sphaerococcus, Ulva, Delesseria and Polyneura. However exceptions were frequent, and the shallow-growing Rhodymenia posessed certain "shade-plant" characteristics whilst the sublittoral Odonthalia appeared to posess certain "sun-plant" ones.

Although the role of the L.hyperborea canopy was not fully investigated, the fact that photosynthesis was less reduced by it in Dilsea than in Ulva, suggested that the light regime beneath the canopy might favour the pigment system of the red algae. Apart from canopy effects, however, in terms of photosynthetic rates, there was evidence of chromatic adaptation in relation to depth in British coastal water : at Puffin Island, Enteromorpha and bleached specimens of red algae showed greater reduction of photosynthesis when transferred to deep sites than did red-coloured red algae from the shallows. However, the photosynthesis of Ulva (and Entermorpha at Puffin Island) was generally quite substantial at deep sites (no canopy present) both in Britain and at Ganzirri, and chromatic adaptation cannot be accepted

as an exclusive mechanism of competition and success in the red algae. In fact, the generally high photosynthetic rates attained by shallow algae when transferred to deep sites (compared with the normal deep ecotypes) suggested that the control and limitation of photosynthesis by irradiance was probably not the sole mechanism of underwater zonation. In this connection, Kain (1966) concluded that competition between L.hyperborea and the fast-growing annual Saccorhiza polyschides was due to factors other than irradiance.

In the present work, there was no indication that deeper algae had lower basal respiration rates (see rates for Peyssonelia, Pseudolithophyllum, Nitophyllum, Tables 8.1 and 3) and in fact the highest photosynthesis : respiration ratio was attained by Rhodymenia from the upper sublittoral.

It is thus not clear what competitive factors limit the colonisation of species such as Rhodymenia and Porphyra to the shallow and littoral zones at Puffin Island. Kain (1960) has recorded these species at 11 and 8m below ELWS at the Isle of Man, however, so they are clearly capable of surviving physiologically, at depths.

One ecological factor which may well have important effects in physiological terms, but is not well documented in this respect, is that of water movement and its effect on the supply of Blackman's (1905) "supply of material". Bidwell (1974, p.592) laid stress on the importance of competition between plants for the "raw materials of photosynthesis". The experiments in Chapter 3 suggested that water movement in the sea might be an important feature in controlling solute supply to the seaweeds, and it was suggested that this supply decreased with depth and high densities of algal stands. It may be, then, that deep -growing species are adapted to conditions of low supply, a situation noted in Delesseria sanguinea and Phycodrys rubens by Schwenke (1971).

In the present work, most of the determinations of photosynthetic rate were made in the summer months, whereas clearly, physiological mechanisms of competition can be expected to act throughout a plant's growth period. Thus, it could be that early in the growing season (winter-spring in Britain), sublittoral conditions may be less favourable to early growth stages of shallow species and thus competition may favour the sublittoral species in a way which is less evident once the community structure has become established in early summer. The in situ experiments at Ganzirri in April showed that the photosynthetic rates of Sphaerococcus and Ulva were some five times higher than when measured in September, which is some evidence of this type of seasonal change. The plant material of Ulva and Porphyra used in April at Ganzirri consisted of sporeling plants of the order of $2cm^2$ total area, and again, this suggests that the relative growth rates of different ontological stages of algae may be widely different. In Britain, photosynthetic rates measured in the summer were quite high, compared with the findings of Jupp (1972), Kain et al.(1975) and Drew (pers. comm.) that L.hyperborea, for instance, is close to compensation under all conditions of irradiance, after May. The physiology of sporeling, adult and senescent stages of marine macroalgae remains an interesting area for further research.

However, regardless of sporeling growth rates and high growth rates of new tissue in spring, in a stratified "climax" forest of L.hyperborea, the underflora of smaller algae must remain non-dominant simply due to their small stature. In this respect the underflora is analagous to that occurring in terrestrial forests, where shade plants of the underflora fill niches less suitable for the canopy-forming trees. In re-colonisation experiments in the sublittoral zone, Kain (1976) found that smaller algae of the Rhodophyta and Chlorophyta could dominate cleared areas for short periods, depending upon the season, but L.hyperborea or Saccorhiza polyschides always succeeded

ultimately. Kain (1969) showed that Saccorhiza could grow in stature, in early sporeling stages, faster than L.hyperborea L.digitata or L.saccharina, due principally to larger cell size. Such a mechanism would not necessarily require higher photosynthesis, or RGR, and could result in greater success in competing for light.

Compensation irradiances calculated from summer photosynthetic rates were quite low in certain algal species, like Porphyra and Rhodymenia (Tables 8, 9 and 10) implying that their compensation depths might correspondingly be quite deep. However, if earlier sporeling stages had different light requirements, these would take precedence in influencing the colonisation pattern. In this connection, Boney (1965) stated that the Conchocelis stage of Porphyra had a very low compensation irradiance and was found in greatest abundance at 32m depth. Again, this seems to suggest that factors other than irradiance confine Porphyra umbilicalis to the littoral zone.

One important environmental factor, other than light, under seasonal control, is temperature. Kniep and Harder (cited by Luning 1971) postulated that low temperatures would result in lowered respiration rates whilst having little effect upon photosynthesis, as suggested by their experimental findings using low (therefore limiting) irradiance levels. They suggested that this would result in peak productivity occurring in winter due to the low respiratory loss in that season. At higher irradiances however, temperature does influence photosynthesis, by altering the height of the plateau of the photosynthesis - irradiance curve. Since high temperatures and irradiance are positively correlated, occurring in summer, it would appear that, other factors being equal, this season is most favourable for algal growth. Annual changes in photosynthesis capacity of one algal species, L.hyperborea, have been ascribed to physiological adaptation to irradiance (Luning 1971) and to the changing nutrient status of the bathing seawater (Jupp 1972), rather than to temperature effects. It seems likely that the high temperatures occurring

in the littoral zone during periods of high irradiance can be inhibiting, in terms of Shelford's "Law" of tolerance, to the colonisation by "sublittoral" species, since Biebl (1972) has found that these species usually possess lower temperature tolerances than littoral species.

Considering the possible annual contribution of the underflora algae to the sublittoral primary production, it will be recalled that the standing crop of non-laminarian algae at Eilean Hoan in August, was 140 g dry weight per square metre, at 5m depth. Assuming a RGR of 0.0264 g g^{-1} d^{-1} (as in Chapter 9, p293) for all the species involved, this crop could be attained in a six month growing period, with a starting mass of only 1g dry weight (using equation 9.9) which could well be present at the commencement of the growing season, as perennating material. It is not known what the biomass of the underflora decreases to between seasons, but Kain (1960) remarked that at the Isle of Man, it was generally reduced compared with summer, but substantially the same in species content. The same author (Kain 1976) found 40-170 g fresh weight m^{-2} of red algal material colonising cleared areas in the months November - December, equivalent to approximately 8-34g dry weight m^{-2}.

The above calculation assumes no losses other than by respiration. Mann (1972) has found that laminarian communities in Nova Scotia produce, over one growth season, up to five times their biomass measured at the end of the season, four-fifths of the production being lost by abrasion. Thus the 140 g m^{-2} crop recorded at Eilean Hoan (based on one sample only, in any case) must represent a minimum annual production, and could possible represent five times as much, 700g m^{-2} (produced from 6g of perennating material). However the losses from the underflora community throughout the growing season are again unknown.

Studies have shown that grazing by echinoderms can be very significant in the ecology of L.hyperborea (Jones & Kain 1967) and Macrocystis pyrifera (Leighton et al.1966). In the case of L.hyperborea, Echinus may completely control the lower depth limit of L.hyperborea. However, experimental work has generally shown that invertebrates show a preference for brown as opposed to red, seaweeds, (Leighton & Boolootian 1963; Himmelman & Carefoot 1975) and when red algae are chosen they are usually of the fleshy type, like Gigartina sp. (perhaps Dilsea would be closest to this in the present work) whereas the dominants of the underflora are generally branched (Plocamium, Ptilota) or membraneous (Delesseria,Phycodrys) forms. Palatable or not, Jones & Kain (1967) found that rock areas at 11m under heavy grazing pressure from Echinus ($\sim$5 individuals m^{-2}) supported only four macroalgal species (two Rhodophyta) whereas in areas cleared of urchins, eighteen species arose (including thirteen Rhodophyta). However, in Nova Scotia, Mann (1973) estimated that in a climax laminarian forest, no more than 10% of macroalgae production was eaten directly by herbivores, the remainder passing into detrital food chains. Although no direct observation was made in the present work, of herbivore attrition of algae of the underflora, it must be admitted that these algae do appear to be particularly physically vulnerable to herbivore attack compared with, for instance, the massive L.hyperborea.

Lack of knowledge of the length of the full growing season of the underflora algae restricts speculation as to their production patterns. As stated above, 1g dry weight m^{-2} would, after six months growing at a rate of 0.0284 gg^{-1} d^{-1} produce 140g, but if, as Mann (1972) found, the growing season was eight months, 560g m^{-2} would be produced. If the starting mass was 5g m^{-2} then 2800g m^{-2} could be produced, close to the overall annual production of 2130g m^{-2} estimated for L.hyperborea on the west coast of Scotland by Jupp (1972).

At Ganzirri, in the absence of the canopy of a single species like L.hyperborea, dense stands of smaller algae were formed, and at 15m, an almost wholly red algal community had a standing crop of over 1000g m^{-2}. Although the growing season here could be expected to be longer than in Britain, it is not known what proportion of this crop was annually renewed. In temperate waters, pure stands of the red alga Chondrus cripsus frequently form (Mann 1972a; Taylor 1972), of biomass up to 1200g m^{-2}. The data of Blinks (1955) suggest that the closely related Gigartina could produce 10,000g m^{-2} in a six month growing season. Since this is so much higher than the actual standing crops attained by Chondrus, it may be that continuous attrition as suggested by Mann (1972) is a constant feature of seaweed ecology.

It has been stated that it appears, from estimates of mean compensation depth that the algae investigated in the present study (and perhaps also dominant species like L.hyperborea) are constantly living at or near compensation point on a 24h basis, more especially if one considers individuals existing in canopy shade. This means that these algae must also be continually operating at their own maximum efficiency. This is an unusual situation in the plant kingdom as has been pointed out by H.T. Odum (1956a) and E.P. Odum (1959). Odum & Pinkerton (1955) suggest that "natural systems tend to operate at that efficiency which produces a maximum power output ..... as opposed to the supposition often made that systems tend to run at maximum efficiency". Similarly, Charles - Edwards (1975) suggested that in certain plant processes it might be preferable "to sacrifice efficiency for expediency, given the constraints that exits in the real world". From figure 7.2 it was clearly seen that efficiency in photosynthesis was highest at the low photosynthetic rates (i.e. low power output) below saturation point. Maximum power output

occurs at saturation point in the shade species, and progressively above saturation irradiance in sun species (Figure 7.3). Odum & Pinkerton (1953) went on to state that "Under certain conditions in nature where raw materials are supplied at constant and minimum rates, there may be times where a slower but more efficient organism might have an advantage". In the sublittoral ecological niche, supply of energy (radiation) and perhaps materials (inorganic carbon, influenced by water movement) may be minimal. The algae studied did not have exceptionally high efficiencies, but what was exceptional was that they were probably operating at their own maximal efficiencies for most of the time. Odum & Pinkerton (1953) had postulated however that in a simple system, maximal power output occurs at 50% of maximal efficiency. It is thus suggested here that the sub-canopy niche of the sublittoral zone in temperate waters is an extreme environment and that the algae colonising it do not conform to the rules governing the growth of the majority of terrestrial plants.

The fact that the majority of the underflora species belong to the Rhodophyta is perhaps no coincidence, apart from considerations of chromatic adaptation. On land, forest floors and many other extreme habitats are characteristically colonised by species belonging to phylogenetically primitive groups, the algae, fungi, lichens, bryophytes and pteridophytes. In the natural world, then, primitive plants, of almost negligible importance in terms of biomass, are pushed by competition to the very extreme niches of the environment by the more advanced and successful plant groups which are important in terms of biomass. This is the fate of the phylogenetically primitive Rhodophyta, a group of plants possessing a unique pigment system, which, although utilising almost the entire spectrum of photosynthetically active radiation, does not apparently confer an equivalent amount of competitive advantage.

SUMMARY

1. At a British site, Durness (Eilean Hoan), the underflora of a L.hyperborea forest, composed almost entirely of Rhodophyta, had a biomass of 140g dry weight m^{-2} at 5m depth, comprising 3.6% of the total biomass of the forest, 4530g dry weight m^{-2}. At 12m the biomass was 84g m^{-2}, comprising 11.5% of the total biomass of 645g m^{-2}. At the Mediterranean site, Ganzirri, on the Straits of Messina almost wholly red-algal stands at 15m had a biomass of 1050g dry weight m^{-2}.

2. ^{14}C isotope and dissolved oxygen techniques (Winkler) for measurement of photosynthesis and respiration of small algae were developed for underwater use to 60m in the sea. The ^{14}C method consistently yielded values of photosynthetic rate higher by a factor of two or more, than measurements made using the oxygen technique. Although the oxygen technique measures net photosynthesis and the ^{14}C method something close to gross photosynthesis, the reason for the large discrepancy was not clear, and could be either methodological or physiological.

3. Photosynthetic rates measured under agitated conditions were approximately twice the values measured under static conditions. Photosynthesis of one algal species, Porphyra, was highest in a current flow of 4cm s^{-1}, the rate being 2.5 times that attained in static conditions. It is possible that in situ experiments, conducted without agitation, produced estimates of photosynthetic rate which were approximately one half of the actual rates. It was concluded that water movement is important in the natural environment in supplying essential solutes to attached macroalgae. A computer model of solute uptake by an alga suggested that the maximum rate of supply which could be sustained by diffusion alone, was 4 µgC cm^{-2} h^{-1} in 1h and 2 µgC cm^{-2} h^{-1} in 6h, at which time the boundary layer thickness would be 2cm.

4. In situ experiments at Ganzirri revealed that Spaerococcus and Ulva reached maximum photosynthesis in the middle of their depth range, from 15-30m. In Britain and at Ganzirri, photosynthetic rates attained by algae growing naturally at the deep sites were usually much lower than those attained by algae which grew at the shallow sites; this was attributable to the reduction of availability of photosynthetically active radiation (PAR) due to attenuation by the water column.

Some rates attained using the ^{14}C method in Britain were:

Shallow species	Depth m	Photosynthesis µgC cm^{-2} h^{-1}	Deep Species	Depth m	Photosynthesis µgC cm^{-2} h^{-1}
Porphyra	0	20	Delesseria	18	3.1
Rhodymenia	3	11	Phycodrys	18	1.8
Ulva	3	10	Ulva	18	3.6
and at Ganzirri:					
Porphyra	4.5	18	Peyssonelia	60	3.2
Ulva	4.5	3.6	Pseudolitho-phyllum	60	4.7
			Ulva	53	3.9

Only at Ganzirri was a seasonal effect on photosynthesis clearly detected. In Sphaerococcus and Ulva, rates were approximately five times higher in April than in September. In Ulva, this was associated with a higher specific lamina area (SLA) in April.

5. Photosynthesis-irradiance curves determined for various British species showed that the deep species - Delesseria, Polyneura, Dilsea - had "shade" plant characteristics, whilst shallow species - Porphyra, Rhodymenia - had certain "sun" plant characteristics. There were exceptions, however, in "deep" Odonthalia (source 9m) which had a high saturation irradiance and in Rhodymenia which had a very high efficiency at low irradiance.

Maximum photosynthetic rates and saturation irradiance levels were, using the ^{14}C method:

Shallow species	Saturation irradiance mW cm^{-2} PAR	Saturation photosynthetic rate µgC cm^{-2} h^{-1}	Deep Species	Saturation irradiance mW cm^{-2} PAR	Saturation photosynthetic rate µgC cm^{-2} h^{-1}
Porphyra	>5	≥18	Delesseria	1	11.9
Rhodymenia	3	32	Dilsea	2	22.5
			Odonthalia	>5	≥8.6

and, using the oxygen method :

Rhodymenia	2.0	6.3	Delesseria	1.0	2.6
			Polyneura	0.5	2.6

Compensation irradiances were variable, but within the range 0.15 - 0.75 mW cm^{-2} PAR.

The maximum efficiency recorded was 16.5% for Dilsea at an irradiance 0.5 mW cm^{-2} using the ^{14}C method in the laboratory.

6. Photosynthesis of the green alga Ulva in situ was always equal to or greater than the rates attained by coexisting red algae, contradicting the chromatic adaptation theory. However, shallow algae of the Chlorophyta (Enteromorpha) and bleached specimens (lacking phycoerythryn) of the Rhodophyta showed greater reduction of photosynthesis when transferred to 18m in British waters than did red-coloured specimens of the Rhodophyta from the shallows.

There was some indication that red algae were better adapted for photosynthesis below the L.hyperborea canopy than green algae.

7. Deep specimens of red species (e.g. Delesseria) exhibited photoinhibition of photosynthesis after irradiation by 40-50J cm^{-2} of the PAR component of surface sunlight; photodestruction of pigments was also noted, causing almost total loss of phycoerythrin.

However, "naturally" bleached specimens of certain species, Laurencia, Polysiphonia, Rhodymenia, Dilsea and Delesseria - had photosynthetic capacities almost as high as their red-coloured counterparts; their respiration rates also were similar or somewhat higher. There were changes in the direction of sun adaptation in bleached Delsesseria.

8. Specific lamina area (SLA = cm^{-2} mg^{-1}) was not found to be correlated with depth of growth, but it was positively correlated with size (and age) of algal thallus, thalli of small area having high values. Membranous thalli (e.g. Porphyra, Delesseria) had SLA $\_$ 0.25 - 0.85 cm^2 mg^{-1} whilst the more dense Dilsea had the lowest value (for non-calcified species) of 0.10 cm^2 mg^{-1}.

Both photosynthetic rate and respiration were positively correlated with SLA, the rates increasing with increasing value of SLA. This meant that photosynthesis and respiration, on a dry weight basis, was less in denser, thicker algae (e.g. Dilsea) than in algae with membranous thalli (e.g. Delesseria).

9. Respiration rates were very variable even within individual species. At Ganzirri, respiration rates of Sphaerococcus measured in situ were positively correlated with diurnal fluctuations in temperature. In Britain, in situ rates were not clearly correlated with temperature, due to the wide variation in values. An indication of the order of magnitude of the rates attained is shown by the mean rates, in Britain:

Shallow species	Respiration rate $\mu gC\ cm^{-2}h^{-1}$	Deep Species	Respiration rate $\mu gC\ cm^{-2}h^{-1}$
Porphyra	0.7	Delesseria	0.6
Rhodymenia	0.5	Phycodrys	0.3
Ulva	0.5		

and at Ganzirri :

Ulva (4.5m)	0.17	Peyssonelia	1.75
Ulva (53m)	0.06	Pseudolithophyllum	2.12
		Sphaerococcus	0.13 ($\mu l O_2 cm^{-2}h^{-1}$)

The highest value of the ratio, photosynthesis : respiration, was attained by Rhodymenia, 39, when the ^{14}C photosynthetic rates were considered. Using the oxygen method the maximum value was 20, attained by the same species.

10. In a generalised computer model of the interaction between depth, time of year and photosynthesis, 24h compensation depths were calculated for an alga with a compensation irradiance of 0.02 mW cm^{-2} PAR and dark respiration rate of 0.5 $\mu gC\ cm^{-2}\ h^{-1}$ (similar characteristics were possessed by Rhodymenia and Delesseria). In summer, in Britain, compensation depth could be around 23m in spring, 16m in winter. winter 10m. However, since these depths do not account for canopy shading, they are necessarily maxima.

11. In situ rates of photosynthesis of shallow algae generally represented relative growth rates (RGR) sufficiently high to produce plants of the observed adult size, from spores, in a 6-month period. Rates for deep species in situ, generally were insufficiently high to do this. The low rates in deep incubations may have been due to the static incubation

conditions, or an indication that growth rates were not at their seasonal maximum at the time of measuring. Propogation from perennating material is liable to confer significant advantage over propagation by spores, which have a very low initial mass.

Again, RGR's derived from measured photosynthetic rates could produce the observed standing crops only if there were initial perennating algal stocks, at the commencement of the growing season, of around 6g (dry weight) m^{-2}.

APPENDIX 1

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A SIMPLE FIELD VERSION OF THE WINKLER DETERMINATION OF DISSOLVED OXYGEN

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SUMMARY

A simple version of the Winkler method of determining dissolved oxygen is described. It is convenient for a wide range of field and laboratory studies.

INTRODUCTION

The photosynthetic rates of aquatic plants and the respiratory rates of both plants and animals are readily studied by measuring changes in dissolved oxygen in closed vessels containing the experimental material. Measurements of dissolved oxygen in open aquatic systems such as ponds, lakes, rivers and seas are also important in determining the biological conditions therein. Despite modern developments such as oxygen-sensing electrodes, the Winkler chemical method is still the most precise and reliable routine method for the determination of dissolved oxygen in such water samples, although various contaminants, such as dissolved organic matter and nitrite, can interfere to some extent. Various modifications developed for specific contaminants have recently been reviewed by Phillips (1973) who also points out that the standard Winkler procedure results in oxygen determinations many times more precise than is usually required in biological work.

A Winkler procedure which we have been using routinely in work on photosynthetic and respiratory oxygen exchange in marine and freshwater algae is described below. This procedure, using relatively small water samples (about 30 ml) eliminates the need for large, complex and fragile glassware such as burettes and B.O.D. bottles, whilst retaining a considerable degree of accuracy and reproducibility. The quantities of corrosive chemicals to be taken into the field are likewise reduced, so that a field pack of six 28-ml universal containers of chemicals, a few disposable syringes and an appropriate number of empty 28-ml bottles as sample jars is sufficient for at least fifty oxygen determinations. Because of its simplicity and convenience, it is applicable to a wide range of field and simple laboratory studies. The method uses the usual Winkler chemical reactions, and details of the standard oceanographic procedures are given by Strickland and Parsons (1965) and in various other standard oceanographic texts.

THE METHOD

The Winkler procedures are carried out in extremely robust screw-capped universal containers of nominal capacity 28 ml. A rubber sealing pad in the lid allows injection of

reagents with hypodermic syringes through small holes punched in the metal or plastic lid.

(1) *The water sample* to be analysed may be obtained in one of three ways.

(a) If the experimental material is suitably small, photosynthetic or respiratory experiments can be carried out in the 28-ml bottles themselves, care being taken to ensure that available oxygen in the small volume of water is adequate for the respiratory needs of the material. The bottle need not then be opened prior to Winkler analysis if the initial reagents (I and II) are injected immediately at the termination of the experiment. The presence of plant or animal material in the jars has not been found to interfere with the subsequent analysis provided this is carried out within 48 h of the injection of initial reagents (see Fig. 1).

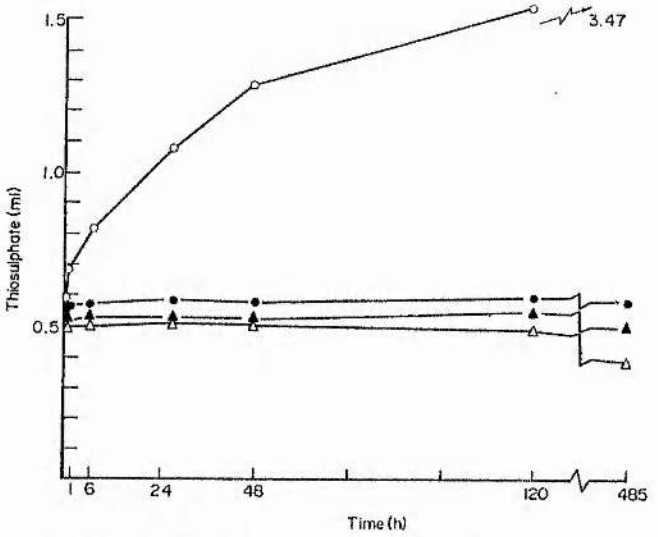

Fig. 1. Changes with time of the apparent oxygen content (thiosulphate titre) in sample bottles under various conditions. ●, Control samples kept in glass jars; ○, control samples kept in plastic (polystyrene) jars; ▲, sample with red algal tissue in glass jar; △, sample with brown algal tissue in glass jar. Reagents I and II injected into all bottles at time zero, bottles shaken and then kept in dark at room temperature for specified times; difference between ● and initial values of ▲ and △ due to presence of live, respiring tissue in bottles for approximately 10 min before injection.

(b) Larger material and longer experiments require larger incubation jars: 1 lb (approximately 475 ml capacity) Kilner preserving jars are often suitable. Subsamples are taken from these jars at the end of the experiment, using 28-ml bottles, as follows. The large jar should be opened carefully to avoid disturbance of the surface layers, and an open 28-ml bottle sunk carefully in; the lid of this should then be screwed on underwater without any air bubbles enclosed (run finger around threads inside lid to ensure this). If difficulty is experienced in manipulating the lid of a 28-ml bottle inside a Kilner jar, then a 28-ml bottle filled to the brim with water from that Kilner jar can be submerged in an even larger container of water and the lid screwed on therein without significant change in the sample.

(c) Water samples can be taken in 28-ml jars from any water source as described in (b) above.

(2) *Fixation of the oxygen* in the 28-ml bottles is carried out by injecting, from a disposable syringe with a large-bore hypodermic needle, 0.5 ml of reagents I (40% $MnSO_4$) and II (50% KI + 50% KOH). A second syringe is used to accept the displaced 1.0 ml of water sample; the reagents are heavier than water and will sink to the bottom of the bottle, displacing only pure water sample. The bottles should then be agitated vigorously for complete mixing and precipitation of the brown manganese hydroxide formed. They can then be left in a cool dark place for 24–48 h, although if dissolved organic matter is suspected to be present, subsequent procedures should be carried out immediately. Living material in the bottles (i.e. in 1(a) above) is killed at this stage by the very high pH caused by the KOH added.

(3) *Analysis of the manganese hydroxide* is carried out when appropriate or convenient by first injecting 0.5 ml of reagent III (50% H_2SO_4) after thoroughly shaking the bottle to re-suspend the precipitate. 0.5 ml of water plus precipitate is lost into the relief syringe; thus a total of 1.5 ml of oxygen-containing water has now been replaced by reagents (because of the 'dilution' of the original samples by reagents I and II, this is nearer 1.48 ml). Vigorous shaking then causes solution of the manganese hydroxide and formation of a golden brown iodine solution, the density of which is an indication of the original oxygen content. The bottle can now be opened and the contents carefully decanted into a 50-ml conical flask; bottles should only be opened one at a time as iodine is volatile and can be lost to the air if the solutions stand open too long.

Six drops of fresh 1% starch solution (S) are added and then the blue iodine complex is titrated with reagent IV (0.05 N sodium thiosulphate). Sufficient accuracy is obtained using a 1-ml disposable syringe graduated in 0.01 ml divisions; the end point is clear under these conditions if working on a white surface. Small quantities of reagent IV (less than 1 drop) can be added near the end point by expelling a small amount on to the hypodermic needle and dipping it into the solution.

(4) *Standardization* of reagent IV, normally made up from prepared volumetric ampoules, is carried out regularly using an accurately prepared solution of KIO_3 (0.10 N = reagent V). Place 25 ml water, 1 ml each of reagents II, III and V and six drops of starch solution in a 50 ml conical flask; then titrate the blue iodine complex with reagent IV as described above. One millilitre of reagent V is equivalent to 2 ml of 0.05 N sodium thiosulphate. The keeping properties of reagent IV in small jars in the dark have been found good, maintaining full strength for several months.

(5) *Calculations* from the titre of sodium thiosulphate used (T in equation) are based on the equivalence of 1 ml 0.05 N thiosulphate to 280 μl of oxygen. The exact volume of the nominally 28 ml bottles should be measured, using either a measuring cylinder or by weighing them full of water and empty.
Thus, μl oxygen originally in bottle =

$$\frac{280 \times T \times V}{V - 1.5}.$$

If the strength of the thiosulphate is different from 0.05 N, the value of 280 should be altered appropriately.

(6) *Reproducibility* of the titration procedure has been verified in a series of twenty con-

trol titrations using seawater taken from a large (3000 l) aquarium tank at 12° C. The value of titre obtained indicated a dissolved oxygen content of 5.78 ± 0.09 ml/l. Seawater saturated with air at this temperature should contain 5.89 ml/l, so that the method appears to give a good estimate of dissolved oxygen.

SUMMARY OF MATERIALS AND REAGENTS

(a) Screw-capped glass universal containers (28 ml nominal capacity) with soft rubber liners in lids and two small holes in lids for injection. Metal or polythene lids are suitable, but plastic bottles must not be used as they are readily permeable to various gases including oxygen as indicated in Fig. 1.

(b) Disposable syringes (1 ml) calibrated in 0.01 ml units, plus large bore hypodermic needles (i.e. 21 G or serum needles).

(c) Large capacity relief syringe plus similar needle.

(d) Conical flask (50 ml) and white tile for titration.

(e) Reagents: I, 40% $MnSO_4$; II, 50% KI + 50% KOH (if brown precipitate forms, decant off clear solution); III, 50% H_2SO_4; IV, 0.05 N sodium thiosulphate (volumetric accuracy); V, 0.10 N KIO_3 (volumetric accuracy); S, 1% soluble starch solution (freshly made). These reagents can all be carried in 28-ml universal containers of the same type as used for determinations.

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```
0000 1-       BEGIN
0001 --       PROCEDURE CILPLT(INTEGER VALUE I); FORTRAN"CILPLT";
0003 --       PROCEDURE CMS;FORTRAN"CMS";
0005 --       PROCEDURE PSPACE(REAL VALUE XMIN,XMAX,YMIN,YMAX);FORTRAN"PSPACE";
0007 --       PROCEDURE MSPACE(REAL VALUE XMIN,XMAX,YMIN,YMAX);FORTRAN"MSPACE";
0009 --       PROCEDURE LIMITS(REAL VALUE XMIN,XMAX,YMIN,YMAX);FORTRAN"LIMITS";
0011 --       PROCEDURE POINT (REAL VALUE X,Y);FORTRAN"POINT";
0013 --       PROCEDURE JOIN(REAL VALUE X,Y);FORTRAN"JOIN";
0015 --       PROCEDURE AXES;FORTRAN"AXES";
0017 --       PROCEDURE FRAME;FORTRAN"FRAME";
0019 --       PROCEDURE IBCINT;FORTRAN"IBCINT";
0021 --       REAL RATE_INTO_1,USE_BY_PLANT,DELTA,TIMESTEP,
0021 --         UPPER,LOWER,
0021 --          UPTAKE,
0021 --          MAX_USE_BY_PLANT,COEFF,CTDD,MUTD,CD,DT,A,B,USE,LAST_USE;
0022 --       REAL ARRAY CONC(1::500);
0023 --       INTEGER NUM_BUCKETS,NUM_TIMES,NB;
0024 --       IBCINT;
0025 --       CILPLT(1);CMS;
0027 --       UPPER:=2.22'-9;
0028 --       LOWER:=1.81'-9;
0029 --       START:
0029 --       NUM_TIMES:=12000;
0030 --       NUM_BUCKETS:=500;
0031 --       MAX_USE_BY_PLANT:=40'-6/3600;
0032 --       LAST_USE:=MAX_USE_BY_PLANT;
0033 --       TIMESTEP:=.2;
0034 --       DELTA:=.0025;
0035 --       COEFF:=2'-5;
0036 --       UPTAKE:=0;
0037 --       FOR N:=1 UNTIL NUM_BUCKETS DO CONC(N):=26'-6;
0038 --       PSPACE(5,30,2,12);
0039 --       LIMITS(0,30,0,15);
0040 --       MSPACE(0,NUM_BUCKETS*DELTA,0,30'-6);
0041 --       AXES;
0042 --
0042 --       PSPACE(5,30,15,25);
0043 --       LIMITS(0,30,0,25);
0044 --       MSPACE(0,NUM_TIMES*TIMESTEP,0,50'-6/3600); AXES;
0046 --       MSPACE(0,NUM_TIMES,0,50'-6/3600);
0047 --       POINT(0,LAST_USE);
0048 --
```

```
0048 --       CTDD:=COEFF*TIMESTEP/(DELTA*DELTA);
0049 --       CTDD:=1-EXP(-CTDD);
0050 --       MUTD:=MAX_USE_BY_PLANT*TIMESTEP/DELTA;
0051 --       CD:=COEFF/DELTA;
0052 --       DT:=DELTA/TIMESTEP;
0053 --
0053 --       FOR T:=1 UNTIL NUM_TIMES DO
0053 2-       BEGIN
0054 --         A:=CONC(1)+(CONC(2)-CONC(1))*CTDD-MUTD;
0055 --         IF A<0 THEN
0055 3-         BEGIN
0056 --           USE:=((CONC(2)-CONC(1))*CTDD+CONC(1))*DT;
0057 --           A:=0;
0058 --
0058 -3         END ELSE USE:=MAX_USE_BY_PLANT;
0059 --         UPTAKE:=USE*TIMESTEP+UPTAKE;
0060 --         FOR N:=1 UNTIL NUM_BUCKETS-2 DO
0060 3-         BEGIN
0061 --           B:=CONC(N+1)+(CONC(N)+CONC(N+2)-CONC(N+1)*2)*CTDD;
0062 --           CONC(N):=A;
0063 --           A:=B;
0064 --           NB:=N;
0065 --           IF CONC(N)>2.599'-5 THEN GO TO OUT
0065 -3         END;
0066 --         OUT:
0066 --         CONC(NB+1):=A;
0067 --         IF NB=NUM_BUCKETS-2 THEN
0067 3-         BEGIN
0068 --           A:=CONC(NB+1)+(CONC(NB)+CONC(NUM_BUCKETS)-2*CONC(NB+1))*CTDD;
0069 --           CONC(NUM_BUCKETS):=CONC(NUM_BUCKETS)+(CONC(NUM_BUCKETS-1)-
0069 --               CONC(NUM_BUCKETS))*CTDD;
0070 --           CONC(NB+1):=A
0070 -3         END;
0071 --
```

```
C001              REAL RIR(100), RII(100), IDD(100), IDI(100), IT(100), R(100),
                 + D(100), T(100), I(100), ZERO(100), INTENS,IMAX,SR(100)
                 + ,MAXI(12),DL(12),DMAXR(25)
                 + ,CARD(10)
0002              LOGICAL WRT
                C
0003            12345 CONTINUE
C004                  IMAX=40
0005                  RMAX=10
0006                  DMAX=40
0007                  SI=2
0008                  NIT=25
                C
0009                  READ(5,100)NRI,CARD
C010              100 FORMAT(I2,10A4)
0011                  READ(5,101)(RII(N),RIR(N),N=1,NRI)
0012              101 FORMAT(2F10.0)
C013                  WRITE(6,200)CARD
0014              200 FORMAT('1   INTENSITY          RATE',5X,10A4)
0015                  WRITE(6,201)(RII(N),RIR(N),N=1,NRI)
0016              201 FORMAT(' ',G14.6,'  ',G14.6)
                C
0017                  READ(5,100)NID,CARD
0018                  READ(5,101)(IDD(N),IDI(N),N=1,NID)
0019                  WRITE(6,202)CARD
0020              202 FORMAT('1   DEPTH        TRANSMISSION',5X,10A4)
0021                  WRITE(6,201)(IDD(N),IDI(N),N=1,NID)
                C
0022                  READ(5,100)NMONTH
0023                  READ(5,101)(MAXI(N),DL(N),N=1,NMONTH)
                C
0024                  CALL GARGS(1)
0025                  CALL CILPLT(1)
0026                  CALL CMS
0027                  CALL PSPACE(5.,20.,5.,15.)
0028                  CALL LIMITS(0.,20.,0.,15.)
0029                  CALL CRSIZE(.3)
C030                  CALL PLOTCS(5.,4.,'RATE VS INTENSITY',17)
0031                  CALL BORDER
```

```
0032                CALL MSPACE(0.,IMAX,0.,RMAX)
0033                CALL AXES
0034                CALL CURVEO(RII,RIR,1,NRI)
0035                CALL FRAME
                C
0036                CALL PSPACE(5.,20.,5.,15.)
0037                CALL LIMITS(0.,20.,0.,15.)
0038                CALL PLOTCS(5.,4.,'TRANSMISSION VS DEPTH',21)
0039                CALL BORDER
0040                CALL MSPACE(0.,DMAX,0.,1.)
0041                CALL AXES
0042                CALL CURVEO(IDD,IDI,1,NID)
0043                CALL FRAME
                C
0044                DO 1000 MONTH=1,NMONTH
0045                DO 10 N=1,25
0046                IT(N)=0
0047                TIME=N-13
0048             10 IF(TIME.GT.-DL(MONTH)/2.AND.TIME.LT.DL(MONTH)/2)
                   + IT(N)=MAXI(MONTH)*(1+COS(6.28318*TIME/DL(MONTH)))/2
0049                WRITE(6,205)MONTH,MAXI(MONTH),DL(MONTH)
0050             205 FORMAT('1MONTH=',I3/' MAX=',G14.6,'   DL=',G14.6)
                C
0051                WRITE(6,203)
0052             203 FORMAT('1   TIME            INTENSITY')
0053                K=0
0054                J=NIT-1
0055                WRITE(6,201)(N,IT(N+1),N=K,J)
                C
0056                DO 3 N=1,NIT
0057              3 T(N)=N-1
0058                CALL PSPACE(5.,20.,5.,15.)
0059                CALL LIMITS(0.,20.,0.,15.)
0060                CALL PLOTCS(5.,4.,'INTENSITY VS TIME',17)
0061                CALL BORDER
0062                CALL MSPACE(0.,24.,0.,IMAX)
0063                CALL AXES
0064                CALL CURVEO(T,IT,1,NIT)
0065                CALL FRAME
```

```
      C
0066        DO 1 N=1,NIT
0067        WRT=(MONTH.EQ.1.OR.MONTH.EQ.4.OR.MONTH.EQ.7).AND.N.LE.13
0068        IF(WRT)WRITE(6,301)
0069      301 FORMAT('0    TIME          DEPTH          INTENSITY       RATE')
0070        DO 2 M=1,51
0071        DEPTH=(M-1)*DMAX/50
0072        INTENS=POLATE(IDD,IDI,NID,DEPTH)*IT(N)
0073        RATE=POLATE(RII,RIR,NRI,INTENS)
0074        T(M)=N-1
0075        I(M)=INTENS
0076        D(M)=DEPTH
0077        R(M)=RATE
0078        IF(WRT)WRITE(6,300)T(M),DEPTH,INTENS,RATE
0079      300 FORMAT(' ',4G14.6)
0080        2 CONTINUE
      C
0081        CALL PSPACE(40.,55.,5.,15.)
0082        CALL LIMITS(0.,55.,0.,15.)
0083        CALL MSPACE(0.,DMAX,0.,RMAX)
0084        IF(N.EQ.1)CALL AXES
0085        IF(N.EQ.1)CALL BORDER
0086        CALL CURVE0(D,R,1,50)
      C
0087        CALL PSPACE(5.,35.,1.,26.)
0088        CALL LIMITS(0.,35.,0.,26.)
0089        IF(N.EQ.1)CALL PLOTCS(5.,0.,'RATE VS DEPTH AND TIME',22)
0090        IF(N.EQ.1)CALL BORDER
0091        CALL MSPACE(-1.5,4.5,-1.5,3.5)
0092        CALL PSPC3D(0.,3.,0.,6.,0.,2.)
0093        CALL CENTER(.5,.5,.3)
0094        CALL VIEW(4.,-6.,3.)
0095        CALL MSPC3D(0.,DMAX,0.,24.,0.,RMAX)
0096        IF(N.EQ.1)CALL AXES3D
      C
0097        CALL CRV03D(D,T,R,1,50)
0098        ZERO(N)=POLATE(R,D,51,0.5)
0099        SR(N)=R(1)
0100        IF(WRT)WRITE(6,300)
```

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